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NOTICE TO SUBSCRIBERS

(Other than Members of the Society)

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ANNUAL GENERAL MEETING

THE eighty-fifth Annual General Meeting of the Society was held at 2.30 p.m. on Friday, March 6th, 1959, in the meeting room of the Royal Society, Burlington House, London, W.1. The Chair was occupied by the President, Dr. J. H. Hamence, M.Sc., F.R.I.C.   The Financial Statement for the year ending October 31st, 1958, was presented by the Honorary Treasurer and approved, and the Auditors for 1959 were appointed.   The Report of the Council for the year ending March, 1959 (see pp. 261–270), was presented by the Honorary Secretary and adopted.

The Scrutineers, Mrs. H. I. Fisk and Mr. P. S. Hall, reported that the following had been elected officers for the coming year—

President—R. C. Chirnside, F.R.I.C.

Past Presidents serving on the Council—J. H. Hamence, D. W. Kent-Jones, J. R. Nicholls and K. A. Williams:

Vice-Presidents—D. C. Garratt, Magnus A. Pyke and J. G. Sherratt.

Honorary Treasurer—A. J. Amos.

Honorary Secretary—R. E. Stuckey.

Honorary Assistant Secretaries—L. Brealey (Programmes Secretary) and S. A. Price.

Other Members of Council—The Scrutineers further reported that 474 valid ballot papers had been received and that votes had been cast in the election of Ordinary Members of Council as follows—C. Whalley, 381; N. L. Allport, 363; F. C. J. Poulton, 317; H. E. Brookes, 305; R. F. Milton, 298; C. H. R. Gentry, 292; H. E. Monk, 272; P. J. C. Haywood, 251.

The President declared the following to have been elected Ordinary Members of Council for the ensuing two years—N. L. Allport, H. E. Brookes, C. H. R. Gentry, R. F. Milton, F. C. J. Poulton and C. Whalley.

R. A. Chalmers, W. T. Elwell, J. Haslam, E. I. Johnson, G. W. C. Milner and T. S. West, having been elected members of the Council in 1958, will, by the Society's Articles of Association, remain members of the Council for 1959.

J. R. Edisbury (Chairman of the North of England Section), A. N. Harrow (Chairman of the Scottish Section), S. Dixon (Chairman of the Western Section), S. H. Jenkins (Chairman of the Midlands Section), F. Holmes (Chairman of the Microchemistry Group), R. A. C. Isbell (Chairman of the Physical Methods Group) and J. I. M. Jones (Chairman of the Biological Methods Group) will be *ex-officio* members of the Council for 1959.

Dr. Williams, the immediate Past-President, expressed the Society's thanks to Dr. Hamence, who had served not only for the past two years as its President, but also for the previous eight as its Honorary Treasurer.   This vote of thanks was carried by acclamation. Dr. Hamence then formally installed Mr. Chirnside in the Chair.

After the business outlined above had been completed, the meeting was opened to visitors, and the retiring President delivered his Presidential Address (see pp. 271–279).

EXTRAORDINARY GENERAL MEETING

AN Extraordinary General Meeting of the Society was held in London on March 6th, 1959, the President occupying the Chair.

A Special Resolution was proposed for altering certain Clauses in the Society's Memorandum of Association, and this Resolution was carried unanimously.

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, May 6th, 1959, in the meeting room of the Chemical Society, Burlington House, London, W.1.   The Chair was taken by the President, Mr. R. C. Chirnside, F.R.I.C.

The subject of the meeting was "The Design of Experiments in Analytical Chemistry" and the following papers were presented and discussed: "The Fundamental Principles of Modern Experiment Design," by E. C. Wood, B.Sc., Ph.D., A.R.C.S., F.R.I.C.; "Statistics in Standardising Chemical Methods," by W. C. Wake, M.Sc., Ph.D., F.I.R.I., F.R.I.C.

NEW MEMBERS

Ordinary Members

Eric W. Atkins, B.Sc. (Lond.); Arthur David Baynes-Cope, M.A., B.Sc. (Dublin), A.R.I.C.; John Bridge; Wilfred Cassidy, B.Sc., A.M.C.T., A.R.I.C.; Charles Bernard Casson, B.Sc. (Lond.), F.R.I.C.; William John Ward Edge, B.Sc. (Manc.); Arthur John Fisher; Robert Rothes Goodall, B.Sc., Ph.D. (Edin.); Frederick Donald Grainger; Narayanaswamy Jayaraman, M.A., D.Sc. (Madras), A.I.I.Sc., F.A.Sc. (Bangalore), A.R.I.C.; Daniel Howe Johns, B.Sc. (Glas.), A.R.I.C., A.M.Inst.F.; John Frederick Kirkland, B.Sc. (Lond.); Eric Blease Lord, B.Sc. (Manc.); David Michael Lodge Morgan; Alan Moss; Peter Norman Robert Nichols; Ann Groot Piken, B.S. (Chicago); Archibald John Pilgrim; Frederick Frank Thomas Purcell; Arthur Eric Purkis; Harold Charles John Saint, A.R.I.C.; Barrie Seaton, B.Sc. (Nottingham); Wynford Lewis Sheppard, A.R.I.C.; John Slater, B.Sc. (Lond.); Arthur W. Spang, B.S. (Wayne State Univ.); Geoffrey Booth Sudworth, B.Sc. (Lond.), A.R.I.C.; William Frederick Swinton, B.Sc. (Lond.); John Jarvis Swithenbank; John Anthony Wilkinson.

Junior Member

Malvern John Thomas Tedds.

NORTH OF ENGLAND SECTION

A Joint Meeting of the North of England Section and the Association of Public Analysts was held at 2.15 p.m. on Saturday, March 14th, 1959, at the Great Northern Hotel, Leeds.   The Chair was taken by the Chairman of the North of England Section, Dr. J. R. Edisbury.

A discussion on "The Composition of Milk" was opened by J. Markland, B.Sc., F.R.I.C.

SCOTTISH SECTION

An Ordinary Meeting of the Section was held at 7.15 p.m. on Wednesday, March 18th, 1959, in the Central Hotel, Glasgow, C.1.   The Chair was taken by the Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C.

The following papers were presented and discussed: "The Estimation of Digestible Carbohydrates in Poultry Foods," by W. Bolton, D.Sc., F.R.I.C.; "A Review of 'Rapid Methods' of Silicate Analysis," by E. L. P. Mercy, Ph.D., D.I.C.

WESTERN SECTION

A Joint Meeting of the Western Section and the South Wales Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Friday, March 13th, 1959, in the Chemistry Department of the University College, Swansea.   The Chair was taken by the Chairman of the South Wales Section, Mr. P. F. Ellis, M.B.E., B.Sc., A.R.I.C.

A lecture on "New Trends in Qualitative Analysis" was given by D. W. Wilson, M.Sc., F.R.I.C.

MIDLANDS SECTION and PHYSICAL METHODS GROUP

A Joint Meeting of the Midlands Section and the Physical Methods Group was held at 6.30 p.m. on Friday, March 20th, 1959, in the Mason Theatre, The University, Edmund Street, Birmingham 3.   The Chair was taken by the Chairman of the Physical Methods Group, Mr. R. A. C. Isbell, A.Inst.P.

The subject of the meeting was "X-ray Fluorescence" and the following papers were presented and discussed: "Instrumental Developments in X-ray Fluorescence Spectroscopy,"

by J. R. Stansfield, M.A., F.Inst.P. (see summary below); "Some Applications of X-ray Spectrography," by H. I. Shalgosky, B.Sc., A.R.I.C.

The meeting was preceded at 2.15 p.m. by a visit to the Research Laboratories of Albright & Wilson Ltd., Langley, Oldbury.

INSTRUMENTAL DEVELOPMENTS IN X-RAY FLUORESCENCE SPECTROSCOPY

MR. J. R. STANSFIELD said that analysis by X-ray fluorescence was both accurate and rapid, and had been applied to the determination of elements in alloys, powders, liquids and other materials.

An instrument for such work included a powerful source of X-rays to irradiate the sample, a crystal spectrometer and a detector to measure the intensity of the radiation emitted by the sample at each characteristic wavelength. These various components and the many different forms that they could take were discussed, and illustrated by descriptions of automatic and manual instruments.

MIDLANDS SECTION

AN additional Ordinary Meeting of the Section was held at 7 p.m. on Monday, March 23rd, 1959, in the Chemistry Theatre, The University, Edgbaston, Birmingham 15. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

Professor Henry Freiser, of the University of Arizona, spoke on some aspects of the analytical applications of chelate compounds.

MICROCHEMISTRY GROUP

THE nineteenth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, April 8th, 1959, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Vice-Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

This meeting took the form of a Review Meeting, at which many subjects related to those already covered in previous meetings were discussed.

SUMMARIES OF PAPERS PRESENTED AT MEETINGS OF THE SCOTTISH SECTION

THE following is a summary of the paper presented by N. T. Wilkinson, F.R.I.C., at the Ordinary Meeting of the Scottish Section on Friday, October 31st, 1958, in Edinburgh. A first report appeared in *The Analyst*, 1958, **83**, 654.

THE ANALYTICAL CHEMISTRY OF PHOSPHORUS

MR. N. T. WILKINSON first dealt with the existing colorimetric, volumetric and gravimetric methods for the determination of phosphorus and described his personal experiences with the early colorimetric procedures. He then discussed the Government Official Methods used in the analysis of fertilisers. The lecturer next gave a detailed consideration of the application of the recently published molybdate - ascorbic acid colorimetric method (D. N. Fogg and N. T. Wilkinson, *Analyst*, 1958, **83**, 406) for the determination of large amounts of phosphorus by means of differential absorptiometry, with special reference to the analysis of fertilisers, and showed that, by comparison with the Government Official Method, the colorimetric procedure was in the class of a referee method. Other applications of the method to the determination of phosphorus in bronze, steel, boiler-water treatment compounds and sugar juices were also discussed and the application of the method to micro analysis was described in detail.

THE following is a summary of the paper presented by F. J. Woodman, B.Sc., A.R.I.C., at the Ordinary Meeting of the Scottish Section on Friday, February 27th, 1959, in Edinburgh. A first report appeared in *The Analyst*, 1959, **84**, 197.

RECENT ADVANCES IN THE ANALYTICAL CHEMISTRY OF PLUTONIUM

MR. F. J. WOODMAN outlined the impact on the analyst of some of the practical problems associated with plutonium chemistry, including the need to work with very small samples, which called for semi-micro techniques, and the existence of a radiotoxic

hazard, which made the use of glove-box methods mandatory. He went on to describe work carried out in recent years in the analytical laboratories of the U.K.A.E.A.'s Industrial Group Works at Windscale.

The speaker first gave the background to the important determination of plutonium at microgram levels in irradiated fuel-element solutions, and described the development of a non-radiometric method involving solvent extraction in the presence of a long-chain quaternary ammonium salt.

He summarised the difficulties of estimating plutonium in urine and other biological materials at the micromicrogram level, and outlined one method that had been worked out at Windscale; this depended on direct precipitation and made use of an alpha-counting finish. He also described a recent very sensitive and specific American method using electro-deposition and an auto-radiographic finish.

Mr. Woodman next outlined a paper-chromatographic technique for the determination of plutonium valency states in solution; this included separations of plutonium^{III} from plutonium^{IV} and of plutonium^{IV} from plutonium^{VI} and was particularly useful for very small samples where spectrophotometry was difficult or impossible.

Recent work on the gravimetric assay of pure concentrated solutions had shown that plutonium sulphate (recommended by earlier workers) was not acceptable, and had established for the first time the optimum ignition and weighing conditions for plutonium dioxide.

Finally, the speaker outlined some applications of the Conway diffusion technique to the determination of nitrogen, chlorine and sulphur in plutonium, and stressed some advantages of the method.

Obituary

GEORGE MACDONALD BENNETT

GEORGE MACDONALD BENNETT, Government Chemist, died suddenly at his home at Worcester Park on the 9th February, 1959, at the age of 66.

He was born on October 25th, 1892, the second son of the Rev. J. E. Bennett, a Baptist Minister, and received his early education in the private school established by his father. The school was later continued by Harold Picton, and Bennett thus in his early years came under the influence of one of the pioneers of colloid chemistry. His brilliant gifts later showed themselves when at East London College (now Queen Mary College) he simultaneously read for both an arts and a science degree, taking his B.A. in 1911 and his B.Sc. with first class honours in 1912.

In 1913 he went as an exhibitioner to St. John's College, Cambridge, where his studies included mineralogy and crystallography as well as chemistry and physics. He developed a lasting interest in crystal measurement and had a first-hand knowledge of liquid crystals, on which subject he could give a fascinating illustrated lecture. He took a first class in both parts of the Tripos and was elected a Fellow of his college in 1917. During the 1914–18 war he worked with Professor W. J. Pope (later Sir William) on mustard gas.

After the war Bennett was for a short time in an industrial post and later went as a demonstrator at Guy's Hospital Medical School. In 1924 he went to Sheffield University as lecturer and in 1931 was appointed to the Firth professorship in chemistry. He came to King's College, London, in 1938 as Professor of Chemistry, and his period here included the war years and evacuation to Bristol.

His interests in chemistry ranged over many fields, among which may be mentioned surface tension, isomerism, stereochemistry of sulphur compounds and glycols, mechanism of nitration and crystallography.

On the death of Sir John Fox he accepted, in 1945, the post of Government Chemist, and devoted his remaining years to the administration of a government scientific establishment. This post he filled with distinction, and he served on a number of official bodies, notably the Royal Commission on Awards to Inventors. He still retained his connection with the academic world and continued to serve as an examiner.

Bennett was elected a Fellow of the Royal Society in 1947 and was appointed C.B. in 1948. He held the degrees of M.A. and Sc.D. at Cambridge and B.A. and Ph.D. at London.

He was an examiner for the Royal Institute of Chemistry from 1944–49, a member of Council from 1949–51 and a Vice-President from 1951–53. He served the Chemical Society as a member of Council from 1929–32 and was an honorary secretary from 1939–46 and a Vice-President from 1948–51. He also served on the Board of Directors of the Bureau of Abstracts.

He married in 1918 Doris Laycock, a Cambridge graduate and a brilliant classics scholar, and he was deeply affected by her death in 1957. They had no children.

Bennett was widely read and the possessor of great gifts, but he was humble and kindly by nature and his staff at the Government Laboratory will remember him with affection not only as their Head, but as a friend.

E. H. NURSE

Annual Report of the Council: March, 1959

DURING the past year the many activities of the Society have shown a general increase, reflecting the increasing importance of all branches of analysis. The widening scope of the methods used, particularly in industry, has had the attention of Council, which has been considering how best to shape the future of the Society so as to take full advantage of this increase in analysis and adequately to cover new techniques.

The provision of meetings has always been one of the most important of the Society's activities and in order to ensure that the meetings should adequately cover modern analytical methods as well as older classical analysis, Council has set up a Programmes Committee. This Committee, with Dr. J. Haslam as Chairman and Mr. L. Brealey as Secretary, met for the first time in April, 1958, and has now become responsible for the organisation of all main Society meetings. It is hoped that this Committee will be able to cover analysis in the widest possible sense. A meeting of particular interest was that held in October, 1958, when Mr. T. T. Gorsuch presented a lecture describing his work for the Analytical Methods Committee to an audience of over 300 at the Royal Institution. It is the policy of the Society to hold joint meetings with other learned Societies on topics of mutual interest, and two particularly well attended meetings were held during the year; one in May with the Association of Clinical Biochemists, the other in December with the Association of Public Analysts.

Although the reports of the Midlands Section and of the Microchemistry Group, given separately, refer to the International Symposium on Microchemistry held in Birmingham in August, 1958, it is appropriate to mention it here. The organisation of the Symposium involved a very considerable amount of work extending over two years and was brought to a successful conclusion in the Symposium where some 60 lecturers and 400 delegates took part. Lectures occupied over 45 hours, covering the widest fields of micro-analysis, and both the scientific and the social programmes were much appreciated. The Executive Committee, under the Chairmanship of Mr. J. R. Leech with Mr. W. T. Elwell as Honorary Secretary, and all those involved, must be heartily congratulated.

The Analytical Methods Committee has now become well established, and it is most gratifying to note the immediate success of its first main publication, "Recommended Methods for the Analysis of Trade Effluents." Among the activities of the Committee that deserve special comment are the initiation of a Committee to examine the determination of additives in animal feedingstuffs, at the request of the Scientific Sub-Committee of the Ministry of Agriculture, Fisheries and Food, and the re-constitution of the Pesticides Residues Sub-Committee. One of this Sub-Committee's first tasks is to initiate research work on non-specific biological tests for residues—a project which is to be financed from the Analytical Methods Trust Fund.

The Annual Conference of Honorary Secretaries of Sections and Groups was held on May 2nd, 1958. The meeting is always of value in that the Honorary Secretaries can meet and discuss questions of mutual interest; this year in particular the Conference was interested in Section boundaries, especially in those Sections where there are considerable travelling difficulties. The Honorary Secretaries undertook to obtain the views of their Sections and the question will again be reviewed.

During the year Council appointed a small Committee to consider *The Analyst* in its broadest aspects. The Society's finances are largely linked with *The Analyst* and with

Analytical Abstracts, and these journals are therefore a matter of considerable importance; their popularity continues to increase, particularly in the U.S.A., and steps are being taken to increase sales in that country. One of the recommendations of this Committee was that a change should be made in the cover of *The Analyst*; accordingly a new cover was commissioned and made its appearance in January, 1959. A new cover for *Analytical Abstracts* also appeared at the same time.

For some years past it has been the duty of the Society to thank the Chemical Council for grants made towards the cost of publication of the Society's journals. In last year's Annual Report the hope was expressed that the Society would be able to meet the cost of its publications. This has now been realised and for the first time for a number of years the Society's publications have shown an excess of income over expenditure. The Chemical Council have recorded their appreciation of the Society's efforts in this direction.

Council records with pleasure the award of the C.B.E. to Dr. R. Lessing and Mr. E. H. Nurse, and of the O.B.E. to Dr. J. B. M. Coppock.

The Society now has 1917 members, an increase of 17 over the membership of a year ago. During the year Council has made arrangements to speed up the approval of new applicants for membership by holding special meetings during the summer; this avoids the period of waiting formerly experienced by candidates applying in May and June.

LONG MEMBERSHIP—The congratulations and good wishes of the Council are extended to J. C. N. Eastick, F. S. Fowweather and W. B. Pollard, who have completed 40 years of membership.

DEATHS—The Council regrets to have to record the deaths of the following members—

J. L. Baker	J. Hawthorne	A. Scholes
A. H. Bennett	E. Hucknall	W. M. Seaber
H. J. Evans	D. C. Macpherson	W. L. Sutton
A. G. Francis	H. M. Mason	J. R. Walmsley
D. M. Gangolli		

ORDINARY MEETINGS—Seven ordinary meetings of the Society were held during the year and the following papers were read and discussed—

April, 1958, in London, organised by the Physical Methods Group, on Gas Chromatography:

Introductory Talk. By C. S. G. Phillips, M.A.
"Applications of Gas Chromatography in the Halogenated Hydrocarbon Field." By R. Hill, B.Sc., A.R.I.C.
"Gas Chromatography in the Petroleum Industry." By D. H. Desty, B.Sc.

May, 1958, in London, Joint Meeting with the Southern Region of the Association of Clinical Biochemists, on Electrophoresis in Clinical Biochemistry:

"Basic Principles and Main Requirements for Satisfactory Electrophoresis." By Professor Nicholas H. Martin, M.A., B.Sc., B.M., M.R.C.P., F.R.I.C.
"Paper Electrophoresis of Lipoproteins." By W. G. Dangerfield, Ph.D., M.R.C.S., L.R.C.P.
"The Clinical Application of Electrophoresis." By A. L. Latner, M.D., M.Sc., M.R.C.P., F.R.I.C.

October, 1958, in London:

Introduction on Radiochemical Methods. By R. Spence, C.B., Ph.D., D.Sc.
"A Report on the Use of Radiochemical Methods to Investigate the Recovery of Trace Elements from Organic Materials." By T. T. Gorsuch, B.Sc., A.R.I.C.

November, 1958, in London:

"Volumetric Analysis of Stannous and Total Tin in Acid-soluble Tin Compounds." By J. D. Donaldson, B.Sc., and W. Moser, B.Sc., A.R.I.C.
"Modification to the Unicam SP500 Spectrophotometer for Single-beam Recording." By D. D. Shrewsbury, B.Sc., Grad.Inst.P.
o-Dithiols in Analysis. Part VIII. The Use of the Zinc Complex of Toluene-3:4-dithiol in the Field Testing of Ores and Minerals." By R. E. D. Clark, M.A., Ph.D., and C. E. Tamale-Ssali.
"The Micro-determination of Calcium and Magnesium in Blood Serum and Cerebrospinal Fluid." By G. Hunter, M.A., D.Sc., F.R.S.C.

November, 1958, in Manchester, organised by the North of England Section:

"The Chemical Examination of Textiles." By J. M. Bather, M.Sc.

December, 1958, in London, Joint Meeting with the Association of Public Analysts, Symposium on Food Analysis: Afternoon Session:

"The Determination of Chemical Antioxidants in Fats after Separation by Partition Chromatography." By K. G. Berger, M.A., N. D. Sylvester, M.Sc., F.R.I.C., and Miss D. M. Haines, B.Sc.
"The Estimation of Egg in Certain Foods by Enzymic Hydrolysis of the Phospholipids." By C. B. Casson, B.Sc., F.R.I.C., and F. J. Griffin, B.Sc., A.R.I.C.

Evening Session opened by J. R. Nicholls, C.B.E., D.Sc., F.R.I.C.:

Two papers under the general heading "The Identification of Coal Tar Colouring Matters in Food Stuffs." By P. S. Hall, B.Sc., F.R.I.C., and R. C. Spalding, M.A., A.R.I.C.

February, 1959, in London, on the Estimation of Pesticide Residues:

Introductory Talk. By G. L. Baldit, B.Sc., A.R.I.C.
"The Determination of Residues of Systemic Organo-phosphorus Insecticides in Vegetables." By E. Q. Laws, B.Sc., F.R.I.C.
"The Determination of Residues of Aldrin, Dieldrin and Endrin." By J. G. Reynolds, F.R.I.C.
"The Determination of Organo-mercury Residues in Plant Material." By M. G. Ashley, F.P.S., F.R.I.C.

NORTH OF ENGLAND SECTION—The membership of the Section is 405, compared with 391 last year. During 1958, eight meetings have been held including the one organised for the parent Society. The following papers have been read and discussed—

Manchester, January, 1958:

"Micro-organisms in Analytical Chemistry." By S. A. Price, B.Sc., F.R.I.C.

Stockton-on-Tees, March, 1958, jointly with the Tees-side Section of the Royal Institute of Chemistry:

"Residues in Foods Deriving from Processing Hygiene and Manufacturing Aids." By J. B. M. Coppock, B.Sc., Ph.D., F.R.I.C., and R. A. Knight, B.Sc., F.R.I.C.

Blackpool, May, 1958, jointly with the Microchemistry Group and the North Lancashire Section of the Royal Institute of Chemistry:

"The Micro Vacuum Fusion Determination of Gases in Metals." By M. R. Everett.
"The Chemical Determination of Nitrogen in Reactor Metals." By J. A. Ryan, A.R.I.C.
"The Determination of Naturally Occurring Radioactive Impurities in a Uranium Purification Process." By M. R. Hayes, A.R.I.C.
"A Simple Manometric Method for the Determination of Carbon in Metals." By M. R. Everett.

Harrogate, June, 1958, Summer Meeting:

"Terylene." By R. J. Gardner.

Liverpool, October, 1958:

Discussion Meeting on "Laboratory Balances," opened by J. G. Lunt, B.Sc., F.R.I.C., and G. F. Hodsman, B.Sc., Ph.D., A.Inst.P.

Sheffield, October, 1958, jointly with the Physical Methods Group and the Modern Methods of Analysis Group of the Sheffield Metallurgical Association:

"The Determination of Gases in Metals by the Micro Vacuum Fusion Method." By E. Booth, B.Sc.
"The Determination of Oxygen and Hydrogen in Steel." By C. E. A. Shanahan, B.Sc., F.R.I.C., F.I.M.

Lancaster, December, 1958, jointly with the North Lancashire Section of the Royal Institute of Chemistry:

"Recent Advances in Polarography and Some Other Electrical Methods." By G. F. Reynolds, M.Sc., F.R.I.C.

SCOTTISH SECTION—During the year Section activities have been carried on in the usual way. Attendances have been somewhat lower than in 1957, but there has been an increase of 5 in membership to the present total of 127. The Proceedings of the 1957 Congress on Analytical Chemistry in Industry held at St. Andrews were published during the current session, the surplus from the Congress making a significant contribution to the cost of printing.

The Section held one meeting in Aberdeen jointly with the Aberdeen University Chemistry Society and the Physical Methods Group; a second meeting was held in combination with the Methods of Analysis Panel (Glasgow). The Society once against supported the Ramsay Chemical Dinner, which continues to be an important function held by the Federation of Chemical Societies in Glasgow. On this occasion the Society was officially represented by the Section Chairman deputising for the President. In addition to the customary meetings, the opportunity was taken of showing the Heyrovský film on polarography. Of the ordinary meetings held, two were arranged in Edinburgh and two in Glasgow. The Annual General Meeting was also held in Glasgow and was addressed by one of the Society's Honorary Assistant Secretaries.

Mr. J. A. Eggleston, who has been Honorary Secretary and Treasurer of the Section during the last eight years, demits office at the end of the present session. The Committee wishes to put on record the great indebtedness to him for the enthusiastic work that he has so consistently done on behalf of the Section.

The following papers have been presented and discussed—

Glasgow, January, 1958, Annual General Meeting:
"Micro-organisms in Analytical Chemistry." By S. A. Price, B.Sc., F.R.I.C.

Edinburgh, February, 1958:
"The Solvent Extraction of Metal Complexes." By F. J. C. Rossotti, B.Sc., M.A., D.Phil.

Glasgow, March, 1958:
Lecture and film on "Polarography." By J. Masek.

Glasgow, April, 1958, jointly with the Methods of Analysis Panel (Glasgow):
"X-ray Fluorescence Techniques in Analytical Chemistry." By E. T. Hall, M.A., D.Phil.

Aberdeen, May, 1958, jointly with the Physical Methods Group and the Aberdeen University Chemistry Society:
"The Analysis of Clays Using Ion-exchange Resins." By Mrs. J. McAuslin, B.Sc., A.R.C.S.
"The Application of Gamma Radiation to the Non-destructive Examination of Coal." By J. Craig Higgins.

Glasgow, October, 1958:
"The Determination of Acidity in Dark Lubricating Oils." By W. Gibb, B.Sc., Ph.D., A.R.C.S.T., A.M.Inst.F., A.R.I.C., and H. Gibson, B.Sc.
"Volumetric Analysis of Stannous and Total Tin in Acid-soluble Tin Compounds." By J. D. Donaldson, B.Sc., and W. Moser, B.Sc., A.R.I.C.

Edinburgh, October, 1958:
"The Analytical Chemistry of Phosphorus." By N. T. Wilkinson, F.R.I.C.

Glasgow, November, 1958, on Developments in Gas Chromatography:
Introduction by A. F. Williams, B.Sc., F.R.I.C.
"Quantitative Analysis Using Thermal Conductivity Detection." By G. R. Jamieson, B.Sc., F.R.I.C.
"The Application of Gas Chromatography to Gas Reaction Kinetics." By J. H. Knox, B.Sc., Ph.D.
"Chromatographic Examination of a Low-temperature Tar." By L. Irvine, B.Sc., Ph.D., A.R.C.S.T., A.R.I.C.

WESTERN SECTION—The membership of the Section is 99, an increase of 14 over last year.

The meetings have been quite well supported by our own members, but, of course, the number of Society members is bound to be small since in any one place there are relatively few members. The policy of having joint meetings with other Societies has been continued with great advantage. The Summer Meeting was very successful with very interesting papers, but the number attending was a little disappointing.

Since the last Annual General Meeting, the following meetings have been held—

January, 1958, Bristol:
Discussion on "Perpetuation of Errors in Technical Books."

February, 1958, Plymouth, jointly with the South-Western Counties Section of the Royal Institute of Chemistry:

"New Techniques in Qualitative Analysis." By D. W. Wilson, M.Sc., F.R.I.C.

March, 1958, Swansea, jointly with the South Wales Section of the Royal Institute of Chemistry:

"Sequestration and its Analytical Applications." By R. L. Smith, B.Sc., Ph.D.

May, 1958, Shrewsbury, Joint Symposium with the Midlands Section on the History of Analytical Chemistry:

"The Origins and Growth of Volumetric Analysis" and "The Origins of Quantitative Organic Analysis." By W. I. Stephen, B.Sc., Ph.D., A.R.I.C.

"The Historical Development of the Public Analyst." By G. V. James, M.B.E., M.Sc., Ph.D., F.R.I.C.

"The Origins of Quantitative Chemical Analysis." By R. E. Coulson, F.R.I.C.

December, 1958, Cardiff, jointly with the Cardiff and District Section of the Royal Institute of Chemistry:

"Recent Trends in Qualitative Analysis." By D. W. Wilson, M.Sc., F.R.I.C.

MIDLANDS SECTION—The membership of the Section is 325, an increase of 9 during the year, consisting of 293 ordinary members and 32 junior members. There are 9 Honorary Members. The principal centres of membership are: Birmingham and District 152, Nottingham and District 57, Derby and District 10, Leicester and District 17, Cambridge and District 18, Bedfordshire 15 and Stafford - Stoke - Crewe 11.

During 1958, twelve ordinary meetings were held; one of these was held jointly with the Birmingham and Midlands Section and one jointly with the East Midlands Section of the Royal Institute of Chemistry. A two-day meeting in Shrewsbury was held jointly with the Western Section. The average attendance at meetings was 50.

A seven-day International Symposium on Microchemistry was held jointly with the Microchemistry Group at the University of Birmingham during the period August 20th to 27th, 1958. This was attended by over 400 delegates from 25 countries.

At the ordinary meetings, the following papers were presented and discussed—

Birmingham, January, 1958:

"The Analytical Chemistry of Nitrogen." By A. F. Williams, B.Sc., F.R.I.C.

Birmingham, February, 1958:

"Nuclear Magnetic Resonance." By D. H. Whiffen, D.Phil.

Nottingham, March, 1958:

"The Analytical Chemistry of Synthetic Detergents." By. W. B. Smith, B.Sc.

Birmingham, March, 1958:

"The Determination of Toxic Substances in the Atmosphere." By J. C. Gage, B.Sc., Ph.D., F.R.I.C.

Birmingham, April, 1958:

"The Analysis of Silicones and Related Organosilicon Compounds." By J. C. B. Smith.

Leicester, April, 1958, jointly with the East Midlands Section of the Royal Institute of Chemistry:

"Developments in the Use of Redox Indicators." By R. Belcher, Ph.D., D.Sc., F.R.I.C., F.Inst.F.

Shrewsbury, May, 1958, Joint Symposium with the Western Section on the History of Analytical Chemistry:

Details of the papers read at this meeting are given in the report on the Western Section.

Birmingham, September, 1958, the following papers were introduced by W. T. Elwell, F.R.I.C.:

"The Volumetric Determination of Chloride in Titanium, Zirconium, etc., Using a Polarisation 'Dead-stop' End-point." By D. Price, B.Sc., and F. R. Coe, B.Sc.

"The Determination of Carbon in Metals, Particularly Titanium and Zirconium, with Special Reference to a Simplified Low-pressure Method." By D. F. Wood, B.Sc., A.R.I.C., and D. A. Williams.

"The Determination of Lead Styphnate in Priming Compositions Used in Explosives." By H. C. J. Saint, A.R.I.C., and Miss J. Hewson.

"The Application of Atomic-absorption Spectrometry in Metallurgical Analysis." By J. A. F. Gidley, B.Sc., A.Inst.P., and J. T. Jones.

Coventry, October, 1958:

Discussion on "The Determination of Trace Impurities in Metals," introduced by B. Bagshawe, A.Met., and W. T. Elwell, F.R.I.C.

Nottingham, October, 1958:

Discussion on "The Identification of the New Permitted Food Colours," introduced by P. S. Hall, B.Sc., F.R.I.C.

Birmingham, November, 1958, jointly with the Birmingham and Midlands Section of the Royal Institute of Chemistry:

"The Infra-red Analysis of Solid Substances." By Professor G. Duyckaerts.

Nottingham, December, 1958:

"The Analysis of Tar Acids." By H. G. Willcock.

Birmingham, December, 1958:

Discussion on "Flame Photometry," introduced by L. Brealey, B.Sc.

MICROCHEMISTRY GROUP—The membership of the Group is now 643, an increase of 25 in the past year.

The Group joined with the Midlands Section in organising the International Symposium on Microchemistry, held in the University of Birmingham on August 20th to 27th, at which 60 papers on microchemical subjects were read and discussed.

During 1958 two ordinary meetings of the Group were held: in London on February 7th (the Annual General Meeting of the Group followed by a meeting of the Society organised by the Group) and in Blackpool on May 16th (together with the North of England Section and the North Lancashire Section of the Royal Institute of Chemistry). The following papers were read—

London:

"Applications of the Conway Diffusion Technique to the Analysis of Radioactive Materials for Trace Impurities." By J. K. Foreman, B.Sc., A.R.I.C.

"The Use of Long-chain Quaternary Amine Salts in the Solvent Extraction of Metal Ions." By R. Powell, A.R.I.C.

Blackpool:

Details of the papers read at this meeting are given in the report on the North of England Section.

Five informal discussion meetings were held in London. The following are the topics discussed, together with the names of the speakers who introduced them—

"Advantages of Spectrophotometric Titrations," introduced by R. A. Chalmers, B.Sc., Ph.D. (jointly with the Physical Methods Group).

"The Micro-determination of Carbon, Hydrogen and Nitrogen in the Presence of Interfering Elements," introduced by G. Ingram, A.R.I.C.

"The Determination of Elements Other than C, H, O, N, S and Cl, Br, I in Organic Compounds," introduced by Miss A. M. G. Macdonald, M.Sc., Ph.D., A.R.I.C., and R. Belcher, Ph.D., D.Sc., F.R.I.C., F.Inst.F.

"The Role of the Microchemist in Industry," introduced by C. Whalley, B.Sc., F.R.I.C., and G. Ingram, A.R.I.C.

"The Use of the Microscope in Analysis," introduced by R. L. Causer, B.Sc.

The Committee met three times during the year. A Sub-Committee dealing with Reagents and Standards in Microchemistry met six times and has produced specifications for 34 standard substances, which it is hoped will be published soon.

PHYSICAL METHODS GROUP—During the past year the Group has held four Ordinary Meetings and also organised the April meeting of the Society. Two of the Group meetings were held in London and one each in Aberdeen and Sheffield. The Aberdeen meeting was held jointly with the Scottish Section and the Aberdeen University Chemistry Society, and the Sheffield meeting jointly with the North of England Section and the Sheffield Metallurgical Association.

Following the Annual General Meeting on November 26th, 1957, the retiring Chairman, Dr. J. E. Page, delivered a lecture entitled "Infra-red Spectroscopy and the Analyst."

The number of Group members is now 708. This is an increase of 51 since the last Annual Report.

The following papers were read and discussed at the Ordinary Meetings of the Group—

Solid Source Mass Spectrometry—London, February, 1958:

"Solid Source Mass Spectrometry—Instrumentation." By G. H. Palmer, B.Sc., A.Inst.P.
"Solid Analysis Using a Spark-source Mass Spectrometer." By R. D. Craig, B.Sc.
"Stable-isotope Dilution Analysis." By R. K. Webster, B.A.

Meeting in Aberdeen—May, 1958:

Details of the papers read at this meeting are given in the report on the Scottish Section.

Determination of Gases in Metals—Sheffield, October, 1958:

Details of the papers read at this meeting are given in the report on the North of England Section.

BIOLOGICAL METHODS GROUP—During the year the membership of the Group has increased from 293 to 307.

In the year ending October 31st, 1958, in addition to the Annual General Meeting, the Group held four ordinary meetings devoted to informal discussion, one demonstration meeting and made one laboratory visit.

Immediately following the Annual General Meeting on December 18th, 1957, a meeting for the informal discussion of "The Weighing and Measuring of Small Quantities" was held jointly with the Microchemistry Group. The discussion was opened by G. F. Hodsman, B.Sc., Ph.D., A.Inst.P., and R. Goulden, A.R.I.C.

February, 1958, London:

Discussion on "The Stage at which a Biological Assay can be Replaced," introduced by W. L. M. Perry, O.B.E., M.D.

Demonstration Meeting, March, 1958, London:

A list of the apparatus demonstrated is given in The Analyst, 1958, 83, 251 (May issue).

April, 1958, London:

Discussion on "The Mathematics of Sterility Testing," introduced by D. Maxwell Bryce, B.Sc., B.Pharm., F.P.S., A.R.I.C.

October, 1958, London:

Discussion on "Strategy in the Assessment of Disinfectants," introduced by G. Sykes, M.Sc., F.R.I.C.

ANALYTICAL METHODS COMMITTEE—The work of the Committee, its Sub-Committees and of the Joint Committee with the Pharmaceutical Society continues to progress satisfactorily and the year has seen an expansion of activity.

The remaining methods for the analysis of trade effluents arising out of the work of the Joint Committee of the Society and the Association of British Chemical Manufacturers were published in The Analyst at the beginning of the year. All the methods were subsequently collected into a single volume, published in July, as "Recommended Methods for the Analysis of Trade Effluents." This book (containing about 55 methods) has proved to be very popular.

The Society would like to take this opportunity of thanking all the members of the Joint Committee for the enthusiastic co-operation and hard work that enabled the methods to be published in such a short time. The Joint Committee has now been disbanded, the Trade Effluents Committee of the A.B.C.M. acting as the standing Committee for future reference.

The other Joint Committee is that of the Society and the Pharmaceutical Society of Great Britain on methods of assay of crude drugs. This has now been in operation for two years during which time a considerable amount of investigational work has been done, and reports are being prepared, by three of the five Panels, on assay of capsicum, lonchocarpus and rauwolfia.

Work continues in the A.M.C. Sub-Committees. A report on the method for lead (a revision of the method published in 1954) is being published in *The Analyst* early in 1959, and a memorandum on the uses and handling of perchloric acid will also appear shortly: both these reports have been prepared by the Metallic Impurities in Organic Matter Sub-Committee. The report by the Vitamin-E Panel on the assay of tocopherols in oils is in its final stages and should be published soon.

Three new Sub-Committees have been appointed by the A.M.C. One of these, on methods for additives in animal and poultry feedingstuffs, has undertaken a large programme of work as a result of a request by the Scientific Sub-Committee of the Ministry of Agriculture, Fisheries and Food. The additives for which recommended methods are required cover vitamins, minerals, hormones and other growth promoters, antibiotics and prophylactics. The Sub-Committee has the status of a steering Committee and is setting up working Panels to cover these five groups of materials. The other two new Sub-Committees have been appointed to prepare methods for pesticides residues in foodstuffs and for chlorine in organic compounds, respectively. The former Sub-Committee is to institute an investigation into non-specific biological tests for the presence or absence of toxic residues.

Mention has already been made of the lecture given by Mr. T. T. Gorsuch at the Royal Institution on the results of his research at A.E.R.E., Harwell, under the direction of Mr. A. A. Smales. Mr. Gorsuch was awarded a scholarship in 1956 by the Trustees of the Society's Analytical Methods Trust Fund to carry out research into the causes of loss (or gain) of trace elements during the destruction of organic matter, using radiochemical techniques for the purpose. The complete report of his work is to be published in *The Analyst* early in 1959.

A separate Report giving full details of the work of the A.M.C. during its fourth year is being prepared and will be circulated to all contributors to the Analytical Methods Trust.

LIAISON COMMITTEE—During the year the following appointments were made—

B.S.I. Committees:

Mr. J. Allen, Colouring Matters for Use in Food Stuffs.
Mr. S. G. E. Stevens and Mr. S. H. Philpot, Essential Oils.

Joint Library Committee, Chemical Society:

Dr. J. G. A. Griffiths was again appointed the Society's representative.

British Iron and Steel Research Association:

Mr. R. C. Chirnside and Dr. J. Haslam represented the Society at the Twelfth Chemists' Conference of the Methods of Analysis Committee (Metallurgy, General Division).

Parliamentary and Scientific Committee:

Mr. G. Taylor continued to represent the Society.

Royal Institute of Chemistry, Summer School Organising Committee:

Dr. J. Haslam and Mr. C. Whalley.

Chemical Council:

Dr. R. E. Stuckey continued to represent the Society.

The Council of the Society thanks all its representatives for the work they have carried out in the various Committees and at varied meetings during the year.

HONORARY TREASURER'S REPORT—The progress made last year toward our aim of being self-supporting, and hence no longer dependent upon grants from the Chemical Council, came to fruition in 1958; each of the publication accounts shows an excess of income over expenditure without the aid of grants, and in the Society's General Income and Expenditure Account also income exceeds expenditure, even before the inclusion of the profits from the publications' accounts. Attainment of this objective has made it possible to plan more surely for the future and to this end the reserves for the decennial indexes have been increased and new reserves initiated against the cost of repairs and decorations of the Society's headquarters and for special publications. The need for this latter reserve is obvious from the accounts; had it not been for the donation from the Scottish Section towards the cost of the Proceedings of the St. Andrews' Congress—a donation for which the Society is most grateful—the difference between the cost of and sales receipts from this special publication, which was nearly £900, would have had to be provided from current income.

Past representations to the Commissioners of Inland Revenue for the Society to be accepted as a charity have failed and accordingly if the Society continues to make a profit it will be called upon for income tax on its annual profit as soon as the losses on previous years have been made good. A new representation will, therefore, be made to the Commissioners after steps have been taken, under Counsel's advice, to meet their previous objections. For the time being, therefore, no reserve against income tax will be created.

While we can be justly proud of our achievement in at last becoming self-supporting our outlook must be one of consolidation rather than rapid expansion; the problem of income tax liability has yet to be settled and we have several heavy commitments ahead. Nevertheless, we can look to the future with optimism.

THE ANALYST—In the early part of the year, Dr. J. R. Nicholls, C.B.E., who had been Chairman of the Publication Committee since 1943 and had served as a member for 20 years, resigned from the Committee. The Council takes this opportunity of thanking him on behalf of the Society for his considerable service in these capacities. Dr. K. A. Williams, Past President, has succeeded him.

The 1958 volume contained 708 pages, compared with 840 in 1957. The numbers of papers and notes published in 1958 were 86 and 53, respectively, against 93 and 52 in 1957. One paper was a Review Paper.

Contributors are backing us up in shortening papers: the average length of papers and notes in 1958 was 4·2 pages, against 4·7 the previous year. We believe that readers prefer a concise presentation, and we think some papers could be shortened further with advantage.

The Publication Committee is actively collecting Review Papers on a wide variety of topics, and it is expected that these will be published at a rate of five or six a year. A continual supply of original papers, too, is always needed from members of the Society and from non-members: the Society repeats the invitation it has extended over so many years to analysts of every country to submit original manuscripts. Readers will already have noticed that a start has been made in publishing descriptions of completely automatic analytical instruments.

Attention is continually being paid to reducing the interval between receipt and publication of papers, and means are still being found for further reductions.

The increasing popularity of *The Analyst* as a medium for carrying advertisements is becoming more and more apparent.

Besides the usual contents of the journal, summaries of 23 papers presented at meetings but not being published in full anywhere were published in the Proceedings of the Society: about half of these were short, consisting of not much more than one paragraph each.

Ten issues of the Bulletin were distributed with *The Analyst* during the year, one of them being a special issue containing the programme of the International Symposium on Microchemistry at Birmingham, organised by the Midlands Section and Microchemistry Group of the Society.

The Society has published three books during the year: in the Spring, the Recommended Methods for the Analysis of Trade Effluents prepared by the Joint A.B.C.M. - S.A.C. Committee appeared collected into book form, as is reported elsewhere, and also the Proceedings of the Congress on Modern Analytical Chemistry in Industry, St. Andrews, 1957. In the autumn the Decennial Index to *The Analyst* for the years 1946 to 1955 was published.

The new cover introduced at the beginning of the current volume has already been favourably received. In a changing world, changes of presentation such as this are to be expected, and it is intended that there should be other changes from time to time.

The number of copies of each issue being printed is 6500.

ANALYTICAL ABSTRACTS—The number of abstracts published in Volume 5, 1958, was 4412 as compared with 4223 in 1957, but the number of pages has not been increased.

The Abstracts Committee has held eleven meetings during the year. Dr. D. C. Garratt resigned from the Committee early in the year and was succeeded by Mr. C. A. Johnson.

The increasing number of abstracts has brought about an increase in the average interval between the date of publication of an original paper and the corresponding abstract. An additional part-time member of the staff, Mrs. K. R. Cook, B.Sc., has been appointed to assist in the preparation and editing of abstracts. It is hoped that a reduction in this time-lag will result.

As a result of a circular letter to a number of organisations and institutions in the U.S.A., seven journals have inserted free of charge a notice relating to *Analytical Abstracts*.

The Abstracts Committee has spent much time in discussing the arrangement of sections in *Analytical Abstracts*. Some minor changes in titles and contents have been made and a fuller explanation of the contents of each section will be given in 1959.

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J. H. HAMENCE, *President.*

R. E. STUCKEY, *Honorary Secretary.*

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Address of the Retiring President

J. H. HAMENCE, M.Sc., Ph.D., F.R.I.C.

(Delivered after the Annual General Meeting, March 6th, 1959)

IT is the duty of the retiring President at the end of his term of office to address the Society and to give—as we may say—an account of his stewardship for the two years during which he has been responsible for the affairs of the Society. Looking back over the Presidential Addresses that have been given by many distinguished Past Presidents of the Society, it is clear that such talks usually take one of two forms. Either the President devotes himself to describing the affairs of the Society during his term of office, or else he gives a learned discourse on some branch of analytical chemistry in which he is particularly interested. I find myself very tempted to adopt the latter course; for, in spite of the fact that, for my sins, I am now very much tied to an office chair, nevertheless I still have a very keen interest in the practical aspects of analytical chemistry, my only regret being that I am unable to take a more active part in its development. One of the tragedies of our age, in my opinion, is that the mass of paper work with which, alas, so many of us are now burdened prevents us from continuing on the more active experimental and practical side of our profession.

In spite of these sentiments, however, I feel it is my duty to devote the major part of this address to the affairs of the Society and to describe in some detail the important events and developments that have taken place during the last two years, and to devote the latter part of the address to a limited number of observations on the course along which I think the future of analytical chemistry should be steered.

Now for the affairs of the Society. It is my opinion that members should know a little more about the inner working of their Society. First, it is my very pleasant duty to pay tribute to the wonderful support that I have received during my two years in office from my two principal Honorary Officers. When I took office two years ago we started as a completely new team, both the Honorary Treasurer, Dr. Amos, and the Honorary Secretary, Dr. Stuckey, being new to their appointments. Both have proved to be towers of strength during this time and are now well seasoned veterans who, I sincerely hope, will steer the affairs of the Society in the same expert and able manner for some years to come. The Annual Report of the Council bears adequate testimony to their work, and I give them my sincere personal thanks for the support they have afforded me.

The last two years have not seen any dramatic or spectacular developments within the Society such as we had three or four years ago, and the work of the Council has largely been concerned with what in Army language may be described as "mopping-up operations" and consolidating our position. (These are the operations that follow a victory and not an accident.) Such a period of consolidation was clearly necessary in view of the many changes that have occurred within the Society during the last decade. During my term of office all the various activities of the Society have been carefully reviewed and discussed by the Council, and where necessary modifications in procedure and design have been made to meet the present conditions.

One of the most important matters that has concerned the Society is finance. The finances of the Society have loomed largely in the minds of the Council since the end of the War. The problem of successive Honorary Treasurers has been to find sufficient funds to enable the Society to develop in a manner in keeping with its dignity and its importance, and to do this on a fairly limited income. This development had to be carried out during one of the most difficult periods in the history of the country's finances; a period in which at times it was almost impossible to keep ahead of ever-rising costs.

Although during this time we were greatly helped by generous grants from the Chemical Council towards the cost of our publications, the policy adopted by the Council has always been that we should so arrange our finances that sooner or later we would become self-supporting. This year the object has been achieved and, as you will have seen from the Balance Sheet presented by the Honorary Treasurer, for the first time in a decade we have balanced all our accounts without the aid of grants from the Chemical Council.

Not only do we thank and congratulate the Honorary Treasurer on this achievement, but we must also thank all those responsible for the production of our Journals who have, in

spite of their ambitions, exercised considerable restraint in their productions so as to keep our expenditure within reasonable limits. I am happy to announce that with the vastly improved condition of our finances we should be in a position to give them more latitude in the future and allow them to develop the Journals along new lines to meet the increasing demands made by analytical chemistry.

Now that we have become self-supporting and obtained a balance on the right side, the question of Income Tax becomes all-important. When the Society changed its name some six years ago it was hoped that we should be exempt from Income Tax, but alas, our hopes were not fulfilled, and the authorities still found clauses in our Articles of Association to which they objected and which were held to be sufficient grounds for still subjecting us to tax.

This meant a still further alteration in our Articles of Association, and we now all sincerely hope that, after the changes that were adopted at the special Extraordinary General Meeting this afternoon, the Commissioners will look favourably upon our request. I personally have always found it difficult to understand why the authorities who profess to be so eager to develop science in this country have raised these difficulties in the past with regard to Income Tax.

Now a word about the Society's office. Dr. Kent-Jones in his Presidential Address in 1955 described the condition of our very modest headquarters in Idol Lane just over ten years ago. It has always been the hope of the Society that sooner or later it would have office accommodation that was not only in keeping with the dignity of the Society but also would afford adequate accommodation for its many different activities.

At the Annual General Meeting in 1957 it was announced that we had moved into Belgrave Square, and at that time we thanked the Society of Chemical Industry for its generous gesture in allowing us to rent the fourth floor of its new headquarters. The office staff, which is now fully installed at Belgrave Square, has a suite of some eight rooms at its disposal, and each of the four sections has its own rooms. We are fortunate enough in having not only office accommodation but also a room that is sufficiently large for Council meetings and which can accommodate the many Committee meetings that are held in the course of the year. This new accommodation has greatly assisted in the carrying out of the ambitious programmes that have been embarked upon in recent years. As many members have not visited us at Belgrave Square, I have had several photographs taken to give you a better idea of our headquarters. These are reproduced on the plate between pages 272 and 273.

We now turn to the various Committees of the Society, many of which are briefly referred to annually in the Report of Council, but few of which have ever been discussed by a retiring President. In my humble submission, insufficient tribute has been paid in the past to those members of the Society who devote a great deal of their time to serving the Society in this way.

First a general word about the constitution of the various Committees, with special reference to regional representation. The Council has devoted considerable time during the last two years to discussions on this matter. This is undoubtedly the result of the rapid development of a virile interest in analytical chemistry all over the British Isles. There was a time when almost the whole of the interests of the Society were centred on London and when meetings in other parts of the British Isles were few and far between. This position has now completely altered, and the same vital interest that led to the initiation of the Society in London some 85 years ago now exists in many other parts of our Islands. This resulted, in the course of years, in the formation of the Sections of the Society, and with the rapid and healthy development of these Sections it is only natural that they should want to take more and more part in the Society's affairs. The big problem confronting the Council is how to give the various Sections of the Society full representation on all its Committees.

No difficulty, of course, arises with the Council itself. The majority of members on the Council are elected freely by ballot by the members of the Society, and apart from this, each Section of the Society has its own representative—either its Chairman or its Honorary Secretary—on the Council. Where the Finance Committee is concerned, arrangements have now been made whereby each Section has a representative on it.

Regional representation is by no means so easy on the two all-important Committees concerned with our publications. In selecting members to serve on both these Committees the primary consideration must, in the opinion of Council, always be given to merit in a particular branch of analytical chemistry and to those members who are in the happy position of being able to devote the necessary time to their allotted tasks. Both these committees

Above: General view of Nos. 14–16 Belgrave Square

Below: The Council Room
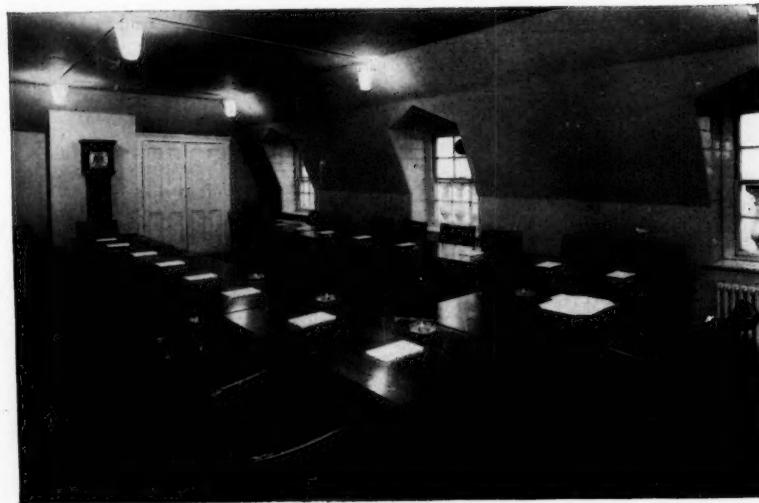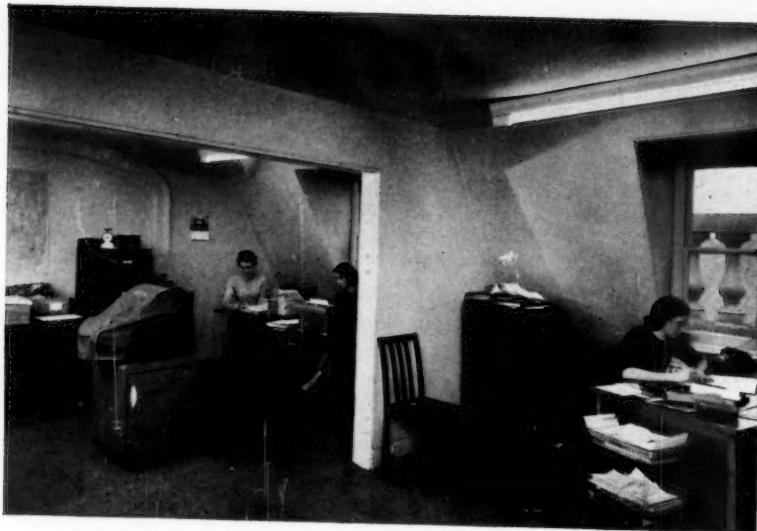
Two of the Society's suite of rooms

involve not only the expenditure of time for attendance at meetings but also a good deal of "homework" in the form of proof reading and the refereeing of papers. This applies particularly to the members of the Abstracts Editorial Committee, who show almost religious fervour in their devotion to the work. The members of both these Committees are specialists who are individually selected to advise on their own particular fields of analytical chemistry. It is in this respect that regional representation runs into difficulties, since it is not always possible to find a suitable specialist from a particular Section of the Society. This difficulty has now been partly overcome by new arrangements whereby representatives from different Sections can attend special meetings of the Publication Committee, so permitting them to express the views of their particular Sections in person. It has also been agreed that when possible members of these Committees shall in future be selected so as to give a better representation of the various Sections of the Society. New blood in the form of younger members is also vitally important on these Committees, and arrangements have now been made whereby this object can be attained.

My two immediate predecessors have both referred at some length in their Presidential Addresses to the strides that have been made by the Society's two principal publications, namely *The Analyst* and *Analytical Abstracts*, and quite clearly our present satisfactory financial position is due in no small measure to the very healthy increase in the sales of these two Journals. I think it is nevertheless a sign of the virility of the Society that, in spite of this apparently satisfactory condition, the make-up of both Journals has been discussed in considerable detail during the last two years, and, indeed, a special Committee was set up with the view to exploring ways and means by which the style or form of *The Analyst* could be improved with a view to increasing its sales.

By now we are all familiar with the new cover of *The Analyst*, which was one of the changes recommended by this Committee; while we hope that you will agree that the general characteristics of the cover have not been lost, nevertheless the style has been brought more in line with modern publications. Review articles are regarded as being very important and steps are being taken to ensure that such articles will in future become a regular feature of *The Analyst*. These articles are by no means new to *The Analyst*, but in the past have been rather few and far between; the Publication Committee is now determined that they should play an ever-increasing part in the make-up of *The Analyst*. One of the major problems in connection with such articles in the past has been to find suitable contributors with the necessary time to prepare them. This is due to the fact that review articles, to be of any real value, must clearly be critical in nature and not merely catalogues or lists of references. To assist in this all-important work Council has now agreed that suitable payment may in future be made to authors of such articles.

Although *The Analyst* is now exported all over the world to some seventy-seven different countries, it is considered that the sales are still unsatisfactory; with this in mind, active steps are being taken to bring *The Analyst* to the notice of a number of American laboratories which at the present time are not subscribers.

Analytical Abstracts like *The Analyst* has now a new cover. From the beginning of 1959 there has been a certain re-arrangement of sections and sub-titles. *Analytical Abstracts* is still pursuing its original policy of abstracting all papers on analytical chemistry, and every year its net is thrown wider to include more foreign journals. In this connection abstracts of as many Russian journals as possible are included.

The year 1958 has seen the formation of a new Committee within the Society, namely the Programmes Committee. For many years the arranging of meetings was left in the good hands of the Honorary Secretary with such advice as he could gather from time to time from the President and Honorary Treasurer. A few years ago this procedure was altered, and the task of arranging meetings and selecting suitable papers was put into the hands of the Publication Committee, it being thought quite rightly that the Publication Committee, which had its finger on the pulse of all new material for publication, was probably the most suitable body to carry out this task.

In view of the fact, however, that meetings now play such a very vital part in the Society's life and of the need for far greater liaison than has hitherto been possible between the parent Society and the Sections and Groups on the subject of meetings, Council decided in 1958 to set up a special Committee devoted entirely to arranging programmes. The major pre-occupation of this Committee is arranging meetings for the parent Society, but its additional function is to assist the Groups and Sections when necessary. This Committee has got off

to an excellent start, and we are fortunate in having Dr. Haslam as its Chairman and Mr. Brealey as its Secretary.

It must be made quite clear that it was never intended that this Committee should interfere in any way with meetings that are normally arranged by Sections and Groups. On the other hand, it is hoped that the new Committee will assist in forming a better liaison between the various bodies and assist in bringing to the notice of the Sections and Groups all meetings that might profitably be duplicated in other parts of the country.

Our Society has now built up a considerable reputation for the quality of its meetings, and attendances are generally very good. The experiment, which was first tried so successfully five years ago, of holding special meetings at the Royal Institution has been repeated twice during the last two years, with equal success. It is a great source of personal pride to me that the principal lecture at one of these meetings was given by a member of our own Society, Mr. Gorsuch, who described, in a masterly manner, the work he had carried out under the direction of Mr. Smales at Harwell to determine the possible losses of trace metals that may occur during the destruction of organic matter.

Our experience has clearly shown that meetings with several papers on one particular subject having a wide appeal are the most successful. On the other hand, "original paper" meetings are still the least well attended. This is disappointing, since before the War the Society relied almost entirely on such meetings, and it is one of the functions of this new Committee to find ways and means of popularising them.

Discussion of original work is still one of the most important functions of our Society, and it is the hope of Council that ways may be found to encourage the originators of new and novel analytical processes to describe their work at meetings of the Society. It is regrettable that far too few of the original papers that have been responsible for the initiation of new analytical techniques have been read before our Society. I would say unhesitatingly to all initiators of new techniques: "Discuss your work before our members, even if the apparatus employed is still only in the string and sealing-wax stage. You can be sure of a receptive and helpful audience. The members of our Society are in a better position than many others to appreciate the possibilities of new techniques and are undoubtedly equally as enthusiastic to try them out." I am firmly convinced that the Society should take a far more active part in moving in this direction and, indeed, the future of analytical chemistry almost inevitably depends upon the development of new techniques to solve the many problems which, in spite of the spectacular advances that have occurred in recent years, still remain to be solved.

The Society is now in great demand for joint meetings with other learned Societies. We encourage such meetings, but clearly, in view of the many meetings already held within the Society's own framework, their number must be restricted.

The Committee also has to bear in mind that there is a strong feeling among the members of the different Sections that more meetings of the parent Society should be held outside London. This is a fair demand and clearly the Society must comply. A start was made in 1958 by holding a meeting of the Society in Manchester, and this must be the forerunner of many more such meetings held in different parts of the country. Groups have always made a practice of holding meetings all over the country. Sooner or later an Annual General Meeting and Biennial Dinner must be held outside London.

While on the subject of meetings mention must be made of the two very successful Congresses held at St. Andrews and Birmingham. Both were proof enough, if indeed any is needed, of the enthusiasm and the organising ability of our colleagues in other parts of the country, and I congratulate heartily all concerned.

Apart from holding meetings of the parent Society in the provinces, there is also a demand that more London members should attend the ordinary meetings of our Sections. The heavy demands made on the Officers of the Society frequently make it difficult for them to attend such meetings, but it is hoped that other London members of the Society, particularly those serving on the Council, may find time to visit the provinces. From personal experience I can assure them all of a most warm welcome.

Finally, we come to the Analytical Methods Committee. I have no doubt that the work of this particular Committee is better known to members of the Society than any other Committee by virtue of the fact that a special full report is published every year of its activities. I hope you all read it. That the work of this Committee is fully appreciated by Industry is evidenced by the very pleasing and generous response by Industry to our second three-year

appeal for funds.   As Treasurer of the Trustees responsible for the administration of the Trust Fund, I am pleased to report that as a result of this second appeal we have been promised sufficient funds to continue our work on the same scale as in previous years.

From my point of view the highlight of the Committee's work during my term of office has been the publication of a joint report with the Association of British Chemical Manufacturers on the Analysis of Trade Effluents.   This report has not only been well received but is also proving to be a chemical "best-seller."   It is interesting to reflect in passing that a number of the newer methods that were worked out in the course of this work have found application not only for the examination of trade wastes, the real purpose for which they were designed, but also to other industrial purposes.

Reference has already been made to the successful meeting at which Mr. Gorsuch described his work on a radiochemical approach to the determination of possible losses of trace elements during the destruction of organic matter.   This work, in my view, is among the most important that has been undertaken by the Society of recent years, for while in some people's minds the work only confirmed what was already known, nevertheless it has reconciled once and for all the many divergent views held by different chemists on this important matter. I think that as a Society we are to be congratulated on having been first to apply a radiochemical technique to this problem, and at the same time we must extend our sincere thanks to Harwell for providing the facilities that permitted this work to be carried out.

It is pleasing to report that several other important projects undertaken by the Analytical Methods Committee are now nearing completion and we await the reports with interest.   One of these is the report of the Vitamin-E Panel, which, apart from describing some novel techniques, gives an account of the first collaborative quantitative paper-chromatographic work to be undertaken.   There are also the reports of the work carried out by the joint committees with the Pharmaceutical Society.   During this work some interesting developments in pure chemistry have been made, reports of which will appear in other journals.

In recent years we have received many requests by different bodies for standard methods, and these bodies include a Government Committee.

The heavy programme of developing the new standard methods of analysis has unfortunately slowed up the work of publishing standard methods, which we were pledged to do in our appeal to Industry.   It has now been agreed that the only way of coping with this situation is to set up a separate Sub-Committee and to engage additional paid secretarial assistance for this purpose.

The annual meeting of the secretaries of Sections and Groups is now a well established function in the Society's affairs.   These meetings prove invaluable in promoting better liaison between all concerned.   One of the problems that has concerned this Committee of recent years is that of Section boundaries, the big problem being—are the present Section divisions the best possible ones from the point of view of meetings?   The problem, of course, is that the railways of Great Britain are largely designed to run up and down the country radiating from London, little attention being paid to cross-country journeys, and in the North there is the additional complication of the Pennines.   This problem is still unsolved and will, we hope, be discussed again at this year's meeting of secretaries.   It is possible that it may eventually result in the formation of another section of the Society.   The Council has also agreed to the setting up of special representatives in the sparsely populated districts to act as liaison officers, and a start has already been made in this direction.   It is also my sincere hope that before long we may see the formation of a local Section in one of the Commonwealth countries.

The function of the Groups has also been recently reviewed.   This review was brought about as the result of the difficulty experienced by one of the Groups in finding suitable material for meetings.   We are all agreed that the formation of Groups at the end of the second World War added very considerably to the prestige of the Society, since it enabled the Society to disseminate knowledge of the newer techniques, which had been developed during the War years and which had passed undiscussed by virtue of the difficulty of holding meetings.   It also enabled the Society to acquire what I like to regard as the "new look" or the new outlook on analytical chemistry.

The early work of the Groups was designed so that teaching was included with the discussion of original techniques.   The point now at issue is that the specialist techniques to which the three Groups devoted themselves have so largely become normal routine operations in most laboratories that they can really no longer be regarded as specialist subjects.

Clearly the Groups will serve a useful function for several years to come, and it is my hope that they may be responsible for bringing within the Society the small groups that have sprung up during recent years for the consideration of specialist techniques. Although this is the position at the present time, I have no doubt that before many years have passed our three Groups will have become re-absorbed into the main body of the Society. To the Group who rather regarded their useful work as having finished, I would only say this: in my view there remains much useful work to be done, particularly along the avenues that are as yet unexplored. Bioassays are beginning to play an increasingly important part in the determination of pesticide residues, and indeed it is my earnest hope that as a result of the work now being undertaken by the Analytical Methods Committee on this subject, they may before long become a routine operation in all laboratories concerned with the examination of foodstuffs. There is also the other aspect that has been developed recently, namely that of growth substances. Bioassays still remain the only method of assessment in some instances. Gibberellic acid is but one example of this, and I put these suggestions forward to the Group concerned for their future consideration.

Having discussed at some length the domestic affairs of the Society with particular reference to the aspects with which Council has been concerned during the last two years, it is appropriate now that I should make a few remarks about the future of analytical chemistry. It is pleasing to record that there is now very clear evidence on all sides that the full importance of this branch of chemistry is being thoroughly appreciated.

I have been considerably heartened during the last two years by the tributes that have been paid to our particular branch of the profession. Dr. J. Craik at the St. Andrews Congress and also Professor Stacey at the Birmingham Symposium both paid generous tributes to analytical chemistry and its importance to industry.

In 1957 the Meldola Medal of the Royal Institute of Chemistry was awarded for the first time for prowess in analytical chemistry. The recipient, Dr. West, a member of the Midlands Section, is also a member of our Council.

It would be quite invidious to compare the respective merits of the different branches of chemistry in an address of this nature, but in the national interest it is vital that the value of analytical chemistry should be fully appreciated. Only by such recognition is it possible to ensure a sufficient number of recruits to this all-important work.

Unfortunately the magic of the word "research" still holds many young students of chemistry rather spellbound, and as a result they prefer to enter a research laboratory instead of the analytical laboratory, which, for some mistaken reason, they regard as being dull and unexciting. Little do they realise that at the present day the analytical laboratory can frequently provide far better outlets for their ingenuity and skill. The remarks of the two distinguished members of the profession mentioned above should go a long way towards dispelling any misunderstandings that may exist in the minds of some of our younger people.

Clearly, apart from man-power, the future of analytical chemistry will depend to some extent upon instrumentation and automation. The papers read at the two Congresses referred to previously show the strides that have been made in instrumentation during the last decade and the benefits, particularly to many branches of industry, which have resulted therefrom. The scope of the work is very wide, and ranges from a study of the reactions occurring in the surface layers of metals in an atomic pile to the production of improved ice-cream.

I sincerely believe that the Society has played a major part in these developments, and it is fitting that we should pay tribute to those members who by their foresight and wisdom made this possible.

The newer techniques that have been made possible by instrumentation are undoubtedly largely responsible for the increase in the prestige of the analytical chemist. Two aspects are here clearly involved. In the first instance the techniques have enabled us to solve many problems which hitherto were regarded as insoluble and to which, alas, the analyst was often in the past unable to suggest any real solution. Chromatography in all its aspects is an excellent example of this. The second aspect of instrumentation is that of speed. Modern industry now relies so largely on scientific control that it is essential in routine control operations to obtain the information required in the minimum possible time. It is in this field particularly that instrumentation has shown its great value. We all know of cases in the past where goods have been sold while samples were still awaiting analysis in the control laboratory. I need only mention the use of the Spekker absorptiometer, and more recently, the Quantometer, in metallurgical work, which now enables the results of an analysis to be obtained in

a matter of hours, and with the latter instrument minutes, whereas before their advent a matter of days was often involved.

Although instruments have proved to be so valuable in the advance of analytical chemistry, nevertheless they should clearly not be mis-used. To my mind there is no point whatever in using a highly complicated and expensive apparatus when a more simple purely chemical technique could give an answer more quickly and quite as efficiently. Bernard Dyer was fully aware of this danger, and referred to it as "chopping blocks with razors."

While undoubtedly as the years advance we shall come to rely more and more on the use of instruments, nevertheless we must not allow instruments to replace human analytical skill. We must still rely on classical chemical methods to provide a basic training for analytical chemists. We hear too often to-day criticism of some of the older classical methods that implies that they give unsatisfactory results, whereas we know that many of these methods in skilled hands have been found to be perfectly reliable in the past. It merely means that the persons responsible for the criticism have not acquired the necessary degree of skill to work the processes satisfactorily. It must be remembered that in modern radiochemical analysis classical methods of separation play a very important part. The skilled craftsman in industry is unfortunately rapidly dying out and is being replaced by the machine. It would be a very sad day if this occurred in analytical chemistry. Instruments are quite impersonal and frequently give little clue of the abnormal or the unusual. We therefore have still to rely upon the skilled analyst to spot these snags by virtue of a kind of "second sight," which can only be developed by years of experience based on adequate training. It is surprising how many of the major discoveries in chemistry have been initiated by some unusual behaviour of a process or by the results of an analysis not adding up as expected.

Although instrumentation is now widely used, it has not been adopted in some branches of analytical chemistry as rapidly as it should have been. A technique such as gas chromatography shows great promise of a very wide application and of replacing what are at the present time rather laborious processes. With this in mind it is pleasing to report that the Analytical Methods Committee of our Society is alive to these procedures and endeavours to give a lead by using them freely in its work. For instance, at the present time the Essential Oils Sub-Committee is applying gas chromatography to its problems.

To some extent I feel that the future is partly in the hands of the manufacturers and that a substantial reduction in the cost of some of the more expensive pieces of apparatus would do much to extend their use. On the other hand, we must always remember that no useful purpose would be served by reducing the cost of a piece of apparatus at the expense of its efficiency.

We now turn to the other aspect of instrumentation, namely automation. Clearly at the present time automation is only in its infancy, but if analytical chemistry is to play its full part in the development of national affairs, far more automation must be introduced.

This requirement is undoubtedly the result of the realisation that most of the problems with which we are concerned are dynamic in nature and not static, as they were so often regarded in the past. While at the turn of the century the analysis of one sample was often regarded as giving sufficient information, now, as a result of our further experience, we realise that only by an examination of a series of samples taken under different conditions is it possible to obtain a true picture of what is happening. These remarks, of course, do not apply in the ordinary way to assay samples.

To cope with this new trend the only hope is automation and the introduction of automatic apparatus. As an example of this, I might quote the work of the Water Pollution Research Laboratory at Stevenage in connection with the dissolved oxygen content of river water that is receiving sewage effluent. This work established the all-important fact that the dissolved oxygen content of river water varies with the time of day, being lowest during the hours of darkness. For obvious reasons it was unlikely that this fact would have been established had it not been for automatic recording equipment. Automatic equipment, although now used in many industrial control processes, clearly needs to be further extended to many routine analytical operations or assays so as to release manpower for more important work.

There was a time when our Society was considered to be too preoccupied with matters relating to food. As a consequence of this we were subsequently inclined to take the Biblical quotation, "Man shall not live by bread alone," too literally, and during the last decade as a result rather to ignore food chemistry. This trend was by no means confined to our own

Society, and a review of the international literature shows quite clearly that the enormous impetus given to inorganic analysis largely as a result of the discovery of atomic energy has resulted in less attention being paid to the analysis of foods. While I fully appreciate the importance of the development of inorganic chemistry as applied to atomic problems, nevertheless I do personally regret the neglect of food problems. In this branch of analytical chemistry to-day there is a great need for the application of the new techniques. Indeed, not only have the newer techniques been applied inadequately, but many more new and yet undiscovered techniques are required to meet the demands made by those who would solve food problems. There is still to my mind a great need for adequate methods of separating and determining natural substances when they are present in a mixture. For too long we have had to rely for such determinations upon one or more of the minor constituents of the natural products. Owing to the variation in composition of natural products it is frequently only possible to arrive at an answer within plus or minus twenty per cent. Much work remains to be done to devise new avenues of attack on these problems.

I was greatly interested in the masterly first Humphrey Davy Memorial Lecture delivered last year by Professor Tiselius. This lecture dealt very fully with the separation of complex organic substances, mainly proteins, by chromatography, electrophoresis and ultra-centrifugal methods. It was shown that by the use of these three different techniques substances that had hitherto been regarded as inseparable by known chemical means were separated quite readily. The question of the separation of blood proteins by electrophoresis was also discussed at the joint meeting of the Society last year with the Association of Clinical Biochemists. Such methods, we understand, are now routine operations in many hospitals.

At the end of his lecture Professor Tiselius dealt with the distribution of relatively large particles of microscopical dimensions between two solvent phases. He showed that by this means separation of different types of organic materials had been achieved, and he finished by saying that the process reminded one somewhat of the flotation methods that had been used for centuries in ore refining, and might be considered as a kind of "biochemical flotation." This process was also referred to in the lecture given by Dr. Synge at the St. Andrews Congress, and he suggested that there was apparently no upper limit to the size of particles that might be separated by this technique. Food chemists will, of course, already be familiar with the technique employed for separating rodent hairs and insect fragments from a large mass of inert foodstuffs by this means.

From the point of view of food chemistry, this new technique should, in my opinion, be explored with the greatest possible vigour. It seems that at last some type of mechanism has been produced whereby the different components of a mixed foodstuff might be separated, and thus a direct estimation of the constituents might become possible. I remember some years ago discussing chromatography, then in a very early stage of development, with a well known chemist, and he remarked "It is a pity you cannot use it to separate the constituents of sausages." This new technique, described in some detail by Professor Tiselius, may in fact permit that wish to be fulfilled and we may in due time be able to separate a mixed foodstuff into its component parts by this means. This would mean a very great advance in food chemistry and would do away with the indirect methods of assessment on which most of our work to-day must inevitably be based.

It may be wishful thinking on my part, but I firmly believe that this new technique offers great possibilities if pursued vigorously enough. On one point I am quite sure—if as a result of further work this promise is not fulfilled, then quite clearly we shall have to look around once more in the hope that someone will have sufficient ingenuity and foresight to develop a new technique that will enable this dream to be fulfilled.

As chromatography is somewhat closely allied to the two-phase separation just described, it may be appropriate to add a general word on the subject at this juncture. I personally still regret the lack of theoretical knowledge of this subject. So often when original papers on chromatography are discussed, particularly paper chromatography, and the author is asked why the particular solvents were used for elution, we are told that it was chosen as the result of a "hit or miss" or "trial and error" technique of using the different solvents, or mixtures thereof, commonly found on laboratory benches. In my view it is a great tragedy that this procedure should still be adopted and that the selection of solvents and of column materials cannot be put on a more scientific basis so that these vital factors could be predicted or selected in advance and not chosen by a "hit or miss" procedure.

It may be appropriate at this stage to say a word about publications. I think we should

congratulate the Society of Chemical Industry upon the pioneer work they are carrying out on documentation. This work is primarily designed to find what information the chemist requires and means of making such information available with the minimum of delay. One of the most disturbing results of this survey is to find the alarming proportions that present-day scientific literature is assuming. It also seems abundantly clear, too, that we still only obtain a portion of the world's scientific literature, and much of the Russian work still remains un-abstracted.

My own immediate reaction to this problem is to ask how much of the vast scientific literature that is published to-day really advances scientific knowledge. With great respect to all concerned, I feel that the answer is: only a small proportion of it. One would hesitate very much to suggest any restriction on scientific publications, but it does seem inevitable that sooner or later something must be done to restrict them to the publication of essential new information and critical reviews only. This is a policy which is now widely adopted in this country, and I should welcome it being more universally applied.

Clearly one of the biggest problems accruing from this ever-increasing literature is that of abstracting, and it is heartening that so far our own *Analytical Abstracts* has managed to keep up with the pace. The biggest problem for *Analytical Abstracts* is undoubtedly indexing; it has now been agreed that the Society should publish a decennial index of analytical abstracts and preliminary work to this end is now in progress. Make no mistake about it, however; this index will be quite a formidable volume.

Finally, ladies and gentlemen, it is my privilege to report that the health of the Society is good and we go from strength to strength.

May I conclude with the words of the poet, Browning, after whom my old School House was named—

"Grow old along with me,
The best is yet to be."

Anniversary Dinner

IN the evening following the Annual General Meeting, a Dinner to celebrate the eighty-fifth anniversary of the Society was held, by kind permission of the Prime Warden, Wardens and Court of Assistants of the Worshipful Company of Fishmongers, at Fishmongers' Hall, London Bridge. The members and guests, numbering 141, were received by the President, Dr. J. H. Hamence, M.Sc., F.R.I.C., and Mrs. Hamence. The President afterwards took the Chair at the Dinner.

The Guests of the Society and of the President included Sir Harry Melville, K.C.B., D.Sc., F.R.S. (Secretary of the Department of Scientific and Industrial Research), and Lady Melville; Professor Sir Charles Dodds, M.V.O., D.Sc., Ph.D., M.D., Hon.Sc.D., F.R.C.P., F.R.I.C., F.R.S.E., F.R.S. (Courtauld Professor of Biochemistry, University of London), and Lady Dodds; Professor H. J. Emeléus, Ph.D., D.Sc., A.R.C.S., F.R.I.C., F.R.S. (President of The Chemical Society), and Mrs. Emeléus; D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C. (Acting President of the Royal Institute of Chemistry), and Mrs. Kent-Jones; A. K. Mills, B.Sc., Ph.D. (Vice-President of the Society of Chemical Industry), and Mrs. Mills; H. E. Monk, B.Sc., M.Inst.S.P., F.R.I.C. (President of the Association of Public Analysts); The Honorable Mr. Justice Lloyd-Jacob, M.A., D.C.L. (Chairman of the Analytical Methods Trust), and Lady Lloyd-Jacob; and F. A. Pester (representing the Worshipful Company of Fishmongers) and Mrs. Pester.

The Loyal Toast was proposed by the President.

Sir Harry Melville proposed the Toast of the Society. He recalled that both at the St. Andrews Congress and the Birmingham Symposium there had been chemists of all kinds, not only analysts. The founder members of the Society, those Public Analysts who had got together to protect themselves and to help check food adulteration, would, he felt, be shocked to find members nowadays who were practically physicists. But analysis now extended into all branches of chemistry, and even theoretical chemists, becoming involved in infra-red spectroscopy, might be eligible as members of the Society. The Annual Report, he noted, listed papers covering all aspects of analysis from nuclear magnetic resonance—almost theoretical physics—to clinical biochemistry.

Sir Harry continued by expressing pleasure that the Society had balanced all its accounts, including the publications accounts, which should excite the envy of other Societies. Since his student days, analytical laboratories had progressed from a few pieces of glassware and a balance to speedy and expensive apparatus, even working automatically sometimes. He had found that the Department of Scientific and Industrial Research tended to become a source of money to some people; one of its tasks was to provide services that individual research organisations could not afford, such as computers.

In conclusion he reminded members of Dr. Pyke's words at St. Andrews, that all wanted to see progress in the growth of analytical chemistry, and that the Society existed to further this progress.

Dr. Hamence, in reply, expressed appreciation of Sir Harry's remarks on the two Congresses held during his term of office. His tributes to analytical chemistry and those of Dr. Craik and Professor Stacey in the introductory addresses at the Congresses had been heartening. He suggested that Sir Harry's reference to the early Public Analysts had perhaps not gone far enough; they had sometimes been called "garblers" because they sorted out the muck and grits. Indeed, many of the great City Companies had been started expressly to maintain quality in this way; certainly the Grocers' Company sorted out spices. He drew attention to the Centenary next year of the first Food and Drugs Act, which would be suitably celebrated. The Public Analysts appointed as a result of that Act started the first analytical laboratories.

Mr. R. C. Chirnside proposed the Toast of the Guests. Analytical chemistry owed something to all of them. Sir Harry Melville, a Meldola Medallist of long standing, had behind him two periods of professorial work and a distinguished war-time career. Now he was Secretary of D.S.I.R.; but still found time to give Christmas Lectures on big molecules to small children. Professor Eméleus was President of the world's oldest Chemical Society. The presence of these two eminent academic chemists at this dinner was in complete contrast to the attitude of the academic world in 1875, which considered Public Analysts as outside the pale. Dr. Mills represented the Society of Chemical Industry, in the absence of Sir Robert Robinson, and through him he thanked that Society for its benevolent landlordship. Dr. Kent-Jones had attended Anniversary Dinners before, first as President of our Society, then as President of the Institute and now, for a reason that was deeply regretted by all, much as he was welcomed for himself, as Acting President of the Institute. It was interesting to speculate in what guise he would appear next time: perhaps it would be as President of the Society of Water Diviners. Mr. Monk was President of the Association of Public Analysts: Public Analysts continued to play an active part in what he hoped they would regard as their "mother Society." Sir George Lloyd-Jacob, besides being Chairman of the Analytical Methods Trust and an Honorary Member, was the 1952 Bernard Dyer Memorial Lecturer, and Mr. Chirnside recalled his pleasure at hearing, and later reading, the beautiful English of the lecture. Sir Charles Dodds was distinguished for his career in biochemistry. His services in this field were not confined to his medical and biochemical colleagues; he had as recently as the previous Friday broadcast on "Laboratories behind the Doctor," telling listeners what doctors and their patients owed to analysis. Finally Mr. Chirnside said how indebted we all were to the ladies for adding to the atmosphere on occasions such as this dinner, for letting us go to Congresses and for coming in increasing numbers to work in laboratories. The Toast of The Guests was coupled with the name of Sir Charles Dodds.

Sir Charles Dodds replied that it was quite true that medicine would not be in the position it occupied today without analytical chemistry. There had been great changes. Dr. Kent-Jones's sphere of work provided another example: in the Middle Ages it was a crime to add chalk to flour; now the reverse was true, and it was an offence for a miller not to put in the statutory amount.

The President concluded the proceedings by investing Mr. Chirnside, the new President, with the Presidential Badge and wishing him a successful term of office. He said that the new President had done much to persuade Council of the extreme importance of the new physical and biological methods of analysis developed during and after the War and so had helped to give the Society its modern outlook. He had also been responsible for the Society's successful venture *Analytical Abstracts*. Mr. Chirnside presented Dr. Hamence with a replica of the Society's badge to wear as Past President.

The Determination of Egg in Certain Foods by Enzymic Hydrolysis of the Phospholipids*

By C. B. CASSON and F. J. GRIFFIN

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A method is described for the determination of egg solids in foods; hydrolysis of the phospholipids is effected by means of the enzyme lecithinase D. The liberated choline is separated by adsorption on a cation-exchange resin and, after elution, is determined colorimetrically as the reineckate.

The enzyme occurs in several vegetables, and the preparation from cabbage leaves of a concentrate sufficiently pure for the purpose is described. The lecithinase concentrate can be conveniently stored at normal air temperature as a freeze-dried powder.

The application of the method to the determination of egg solids in salad cream is dealt with. It is shown that enzymic hydrolysis gives more accurate results than acid hydrolysis, because interference by mustard is reduced. Enzymic hydrolysis has been applied also to the determination of egg in ice-cream and in lemon curd.

THE egg content of food products is of importance to the food technologist, to the dietician and in relation to legal standards. For salad cream and mayonnaise, the legal minimum is 1·35 per cent. of egg-yolk solids[1] and for lemon curd 1 per cent. of whole-egg solids.[2]

The methods chiefly used for determining egg in foods have depended on the determination of characteristic constituents of the yolk, such as cholesterol[3] or phospholipids. As both these constituents occur in other food ingredients (although usually in much smaller proportions), appropriate corrections become necessary when either is introduced from such sources. For phospholipid determinations, the methods used have relied on determination of either the phosphorus associated with the phospholipids (solvent-extractable phosphorus)[4] or the choline. The former methods have been widely used, but may give low results if the phospholipids have become partly hydrolysed during storage of the food product; it has been shown[5] that enzymes in certain foods may catalyse such hydrolyses. During storage, enzymic hydrolysis may also liberate choline from the phospholipid molecule, but this choline normally remains available for determination, so that the hydrolysis is unlikely to affect results by the choline method.

When total choline is determined, the phospholipid molecule must first be completely hydrolysed. Both acid[6,7] and alkaline hydrolysis[8,9] have been proposed. Daubney and Sexton[6] evolved an acid-hydrolysis procedure for salad cream, but found that sinalbin in the mustard (a normal ingredient of salad cream) was also hydrolysed and liberated choline. Unless, therefore, a satisfactory method can be found for independently determining the mustard in salad cream, serious errors may arise in the evaluation of the egg content by acid hydrolysis. Acid hydrolysis is also unsuitable for products containing appreciable proportions of sugar, as the caramelisation products interfere with subsequent separation of choline.

We have used enzymic hydrolysis as a preliminary to choline determination in products of the type mentioned. Lecithinase D, which catalyses the reaction, has been shown by Hanahan and Chaikoff[10] to be present in cabbage leaves, carrots and other vegetables. They gave details of the preparation of enzyme concentrates from these vegetable sources, and, as a result of their work and that of Kates,[11] Einset and Clark,[12] Davidson and Long[13] and others, the optimum conditions for the activity of this enzyme are now well established. The optimum pH is 5·6, a temperature range of 25° to 35° C is suitable and the activity of the enzyme is increased in presence of ether or calcium ions.

EXPERIMENTAL

PREPARATION OF ENZYME CONCENTRATE—

Hanahan and Chaikoff's extract was prepared from cabbage; a puree was made, and the liquor was separated by straining the puree through muslin and then spinning it in a centrifuge.

When tested on a substrate of dried-egg dispersion, the supernatant liquor had considerable enzymic activity, but the choline from the cabbage led to high blank values. The liquor was therefore set aside overnight at about 20° C to ensure complete hydrolysis of cabbage phospholipids. The pH was reduced to 4·9, at which a flocculent precipitate was formed. When separated, this precipitate was found to be active and practically free from choline. Although a suspension of the precipitate in water could be used directly for determining egg, it was found to be more convenient to freeze-dry it and to use a suspension of the powder. From 3 kg of cabbage, the yield of freeze-dried powder has usually been about 10 g; the powder is not hygroscopic and can be kept in the dark for several months without loss of potency.

PRELIMINARY INVESTIGATION—

Hydrolysis of the phospholipids was carried out under the optimum conditions, *i.e.*, at 30° C, pH 5·6 and in presence of ether and calcium ions. When 10 ml of a 1 per cent. suspension of the dried enzyme preparation were used, hydrolysis of the phospholipids was complete in 16 hours, provided that not more than 6 mg of choline were present. Continuous agitation was found to be necessary, owing to the heterogeneous nature of the reaction mixture. Methanol was a suitable precipitant for extraneous material in the hydrolysate; trichloroacetic acid was not satisfactory, as choline tended to be adsorbed on the precipitated material. A cation-exchange column of the sulphonic acid type was adequate for separating choline from the clarified hydrolysate. For the final determination of choline, the practical details of the reineckate procedure described by Daubney and Sexton[6] were found to be satisfactory.

METHOD

PRINCIPAL REAGENTS—

Acetate buffer solution—Dissolve 13·6 g of hydrated sodium acetate in 950 ml of distilled water, adjust to pH 5·6 with glacial acetic acid, and dilute to 1000 ml.

Ammonium reineckate solution—Prepare a 2 per cent. solution of ammonium reineckate in methanol, and filter. This solution should be prepared daily as required.

Standard choline chloride solution—Dissolve enough choline chloride in distilled water to give a 2 per cent. (or slightly more concentrated) solution of choline. Standardise this solution against silver nitrate, and adjust to exactly 2 per cent. of choline. For use, dilute 5 ml to 100 ml; 1 ml of the diluted solution contains 1 mg of choline.

ION-EXCHANGE COLUMN—

Lightly grind some Zeo-Karb 225 in a mortar, and set aside under 5 N hydrochloric acid for 6 hours with occasional stirring. Wash the resin free from acid and fine particles. Place a glass-wool pad at the suitably constricted base of a glass tube, 300 mm × 11 mm (internal), and add the prepared resin to a height of 80 mm. Pass an excess of 3 per cent. aqueous potassium chloride solution through the column, wash with water, elute with 5 N hydrochloric acid, and finally remove the acid with water. Repeat the process twice more to increase the capacity of the resin.

PREPARATION OF ENZYME CONCENTRATE—

Comminute 200 g of white-hearted cabbage leaves and 150 ml of distilled water in a high-speed mixer, *e.g.*, a Waring Blendor, until a smooth puree is obtained. Set the puree aside at about 5° C for 1 to 2 hours, and then filter it through muslin. Pass the liquor through a laboratory basket-centrifuge lined with Whatman No. 54 filter-paper. Set the separated liquor aside overnight at about 20° C. Add 5 N hydrochloric acid dropwise until the pH is between 4·9 and 5·0, as judged by narrow-range test papers. Separate the precipitate by centrifugation for 15 minutes at 1500 g, and decant the supernatant liquor. For immediate use, suspend the residue in a volume of water equal to one quarter of the volume of liquor from which it was separated, and use 10 ml of this suspension for each determination. Alternatively, freeze-dry a large amount of precipitate, and store it in the dark at room temperature until required. Use 10 ml of a 1 per cent. suspension of the freeze-dried powder for each determination.

PROCEDURE—

Weigh into a 100-ml conical flask enough sample (maximum 10 g) to contain not more than 6 mg of choline, add 15 ml of acetate buffer solution and 0·35 ml of M calcium chloride. Thoroughly shake the mixture, use narrow-range test papers to adjust the pH to 5·6, add 10 ml

of enzyme suspension, and re-adjust to pH 5·6 if necessary.   Finally, add 0·6 ml of diethyl ether per millilitre of reaction mixture.   For acid products, such as salad cream, determine the amount of alkali needed to bring the pH to 5·6 by a separate titration, and add this amount as 0·5 N sodium hydroxide.   Prepare blank solutions by using distilled water in place of the sample.

Warm the flasks to 30° C, insert stoppers, and set them aside at 30° C for 16 hours (shake mechanically during this period).   Gently evaporate the ether by standing the flasks in hot water.   Transfer the hydrolysates to 100-ml calibrated flasks with methanol, using a rubber-covered glass rod to remove solid matter from the sides of the flasks.   Cool, and dilute to volume with methanol, taking care to remove air bubbles entrapped in the precipitate.   Filter each solution through a Whatman No. 4 filter-paper into a dry 250-ml conical flask, suitably covering funnel and flask to avoid loss by evaporation.   Pass exactly 80 ml of the filtrate through the ion-exchange column at the rate of 1 drop per second.   Wash the column with four 10-ml portions of distilled water, and elute the adsorbed choline with six 10-ml portions of 5 N hydrochloric acid and then 10 ml of distilled water.   The choline can conveniently be eluted into a 100-ml conical flask that has been etched at 60 ml.   Evaporate the eluate to 10 ml, cool, make just alkaline to thymolphthalein with 30 per cent. potassium hydroxide solution, and then add glacial acetic acid, dropwise, until the blue colour just disappears (about pH 9·3).   Dilute to the 60-ml mark with distilled water.   Note that as much hydrochloric acid as possible must be removed during evaporation, as ammonium reineckate may be salted out later if the solution contains too much potassium chloride.

Prepare a series of standards containing 2·0, 4·0, 6·0 and 8·0 ml of dilute standard choline solution, and dilute each to 60 ml in conical flasks.   Add 6 ml of ammonium reineckate solution to each of the standard solutions, the sample solution and the blank solution, and set aside overnight at 0° to 2° C.   Separate the pink precipitates of choline reineckate on No. 3 sintered-glass filters, and wash each precipitate with three 2·5-ml portions of ice-cold water and then with three 2·5-ml portions of n-propyl alcohol.   Dissolve each precipitate in acetone, place the solution in a 50-ml calibrated flask, and dilute to the mark with acetone.   Measure the optical density of each solution against acetone in 4-cm cells with an absorptiometer and an Ilford No. 604 filter (peak transmission 520 mμ).   Plot optical density against choline content for the standard solutions, and read the choline content of the sample from the curve obtained after allowance has been made for the blank value.

NOTE—For samples containing a high proportion of fat, determine the fat content independently, and make a correction for the volume of fat in the methanol mixture (assume its specific volume to be 1·1).

RESULTS

The method described has been applied to egg in commercial forms, to other phospholipid-containing food ingredients (to permit corrections to be applied) and to laboratory-made composite foods.   Choline contents of the food ingredients have been expressed as choline in dry matter, the total solids having been determined.   The composite egg-containing foods, salad cream, lemon curd and ice cream, were prepared from carefully measured ingredients and, by taking processing losses into consideration, it was possible to calculate the egg contents of the finished products.   The calculated egg contents were compared with the analytical results.

The results in Table I show that choline contents were reasonably constant for whole-egg solids.   The average value, 1·58 per cent., is close to the value recommended by Daubney and Sexton[6] as a basis for calculations, viz., 1·6 per cent., and we have therefore used this value. The choline content of the solids in the sample of egg yolk also agreed closely with Daubney and Sexton's results and is in harmony with the accepted ratio of yolk to white in whole-egg solids.

The separated-milk powders have much higher choline contents than has the butter; this indicates that choline tends to be associated with the solids-not-fat in milk rather than with the fat.   This is in accordance with the findings of Engel,[8] who attributed the high results for separated milk to a close linkage between phospholipid and protein.

Maize oil and margarine were found to contain little choline; similar low results have been reported by other workers.   Unless, therefore, the proportion of these ingredients in a composite food is large, their choline content will have little effect on the calculation of egg solids. Commercial soya lecithin showed a high choline content, as would be expected.

TABLE I
CHOLINE CONTENT OF EGG AND OTHER FOOD INGREDIENTS

Sample				Total solids, %	Amount of choline found, %		Mean amount of choline in dry matter, %
Dried whole egg 1 (U.S.A.)	..	..	..	94·1	1·48,	1·53	1·60
Dried whole egg 2 (Dutch)	..	..	..	95·8	1·56,	1·54	1·62
Dried whole egg 3	..	..	..	95·2	1·60		1·68
Dried whole egg 4	..	..	..	95·3	1·48,	1·53	1·58 Average,
Dried whole egg 5	..	..	..	95·8	1·49,	1·50	1·56 1·58
Frozen whole egg (Chinese)	..	..	..	26·3	0·41,	0·39	1·52
Frozen whole egg (English)	..	..	..	26·2	0·40,	0·40	1·53
Fresh egg yolk	..	..	..	49·6	1·12,	1·11	2·25
Spray-dried separated-milk powder 1	..	..	96·5	0·090,	0·092	0·094
Spray-dried separated-milk powder 2	..	..	95·9	0·105,	0·110	0·112
Roller-dried separated-milk powder	..	..	96·6	0·090,	0·097	0·097
Butter	..	..	..	84·5	0·009,	0·009	0·011
Fresh whole milk	..	..	..	12·7	0·014,	0·015	0·114
Cream (50% fat)	..	..	..	55	0·016,	0·015	0·028
Maize oil	..	..	..	100	0·003,	0·004	0·004
Margarine	..	..	..	84·4	0·005,	0·005	0·006
Commercial soya lecithin	..	..	..	100	2·18,	2·12	2·15
Mustard 1	..	..	..	..	—	0·25, 0·27 (0·67, 0·72)*	—
Mustard 2	..	..	..	—	0·31, 0·32 (0·60, 0·61)*	—
Mustard 3	..	..	..	—	0·31, 0·33 (0·63, 0·62)*	—

* Results by acid hydrolysis.

Table I also shows the choline contents of three samples of commercial mustard; for comparison, results by acid hydrolysis are shown in parenthesis. The choline content by enzymic hydrolysis is about half that found by acid hydrolysis.

Table II shows the results when the method was applied to samples of salad cream and lemon curd. Salad-cream samples Nos. 1 to 5 each contained 1 per cent. of mustard and whole-egg solids ranging from 0 to 3·65 per cent. The choline contents after acid hydrolysis were appreciably higher than those after enzymic hydrolysis. When these values were calculated as whole-egg solids, without correction for mustard, the results were as shown in the last two columns. In the absence of egg, an apparent egg-solids content of 0·23 per cent. was found by enzymic hydrolysis, as compared with 0·41 per cent. by acid hydrolysis. This is a measure of the effect of mustard, and the results are those that would be expected from the values for mustard in Table I. This suggests that no hydrolysis of sinalbin takes place during the pasteurisation of salad cream. We have confirmed this by separate experiments in which mustard was heated with acetic acid. The last two columns of Table II show that, in presence

TABLE II
EGG SOLIDS IN SALAD CREAM AND LEMON CURD

Food	Sample No.	Amount of choline found by—		Amount of whole-egg solids present, %	Amount of whole-egg solids calculated from choline found by—	
		enzymic hydrolysis, %	acid hydrolysis, %		enzymic hydrolysis, %	acid hydrolysis, %
Salad cream containing 1 per cent. of mustard	1	0·0036	0·0065	Nil	0·23	0·41
	2	0·0184	0·0235	0·92	1·15	1·47
	3	0·0323	0·0380	1·85	2·02	2·37
	4	0·0440	0·0475	2·68	2·75	2·97
	5	0·0602	0·0700	3·65	3·76	4·38
Salad cream without mustard	6	0·0592	—	3·65	3·70	—
Lemon curd	1	0·015	—	1·0	0·94	—
	2	0·079	—	5·0	4·94	—
	3	0·150	—	8·9	9·4	—

of mustard, enzymic hydrolysis gives results closer to the true egg content than are those by acid hydrolysis, although the results after enzymic hydrolysis are still slightly high. The three samples of lemon curd gave results in reasonable agreement with the composition of the samples.

Table III shows the results for samples of ice-cream. The whole-egg solids calculated from the total choline are high, which emphasises the need to correct for choline introduced by the milk products. (Before a correction could be applied to an unknown sample, it would be necessary to determine the milk-solids-not-fat and the butter fat present.) A correction (derived from Table I) was then applied on the assumption that butter and separated-milk powder had been used; this gave the results shown at (b) in Table III. These results are satisfactory for sample No. 3, in which only separated-milk powder had been used, but they are slightly high for samples Nos. 1 and 2. Line (c) of Table III shows the egg solids calculated after correction for milk solids in the form in which they were known to be present in the samples. For samples Nos. 1 and 2, which contained fresh milk and cream, this gave a slightly lower egg content.

TABLE III

EGG SOLIDS IN ICE-CREAM

Sample No.								1	2	3
Composition from ingredients used, %—										
Whole-egg solids								1·24	1·62	3·50
Separated-milk powder								8·1	Nil	10·0
Fresh milk								8·8	40·7	Nil
Cream (50% fat)								8·8	40·7	Nil
Sugar								15·5	16·2	14·4
Vegetable fat								9·0	Nil	11·2
Analytical results—										
Total choline found, %								0·0305	0·0365	0·0662
Amount of whole-egg solids, %—										
(a) Calculated from total choline								1·91	2·28	4·14
(b) After correction for choline in milk products (as butter and separated-milk powder)								1·31	1·77	3·52
(c) After correction for choline in milk products (as added)								1·24	1·52	3·52

REFERENCES

1. Food Standards (Salad Cream and Mayonnaise) Order, S.R. & O., 1945, No. 1177.
2. Food Standards (Preserves) Order, S.I., 1953, No. 691.
3. Haenni, E. O., *J. Ass. Off. Agric. Chem.*, 1941, **24**, 119; 1942, **25**, 365.
4. Horwitz, W., *Editor*, "Official Methods of Analysis of the Association of Official Agricultural Chemists," Eighth Edition, The Association of Official Agricultural Chemists, Washington, 1955, p. 215.
5. Acker, L., and Lück, E., *Z. LebensmittUntersuch.*, 1958, **107**, 143; 1958, **108**, 256.
6. Daubney, C. G., and Sexton, G. E. W., *Analyst*, 1950, **75**, 305.
7. Munsey, V. E., *J. Ass. Off. Agric. Chem.*, 1953, **36**, 766.
8. Engel, R. W., *J. Nutrit.*, 1943, **25**, 441.
9. Salwin, H., Devine, M. D., and Mitchell, J. H., *J. Agric. Food Chem.*, 1958, **6**, 475.
10. Hanahan, D. J., and Chaikoff, I. L., *J. Biol. Chem.*, 1947, **169**, 699; 1948, **172**, 191.
11. Kates, M., *Canad. J. Biochem. Physiol.*, 1954, **32**, 571; 1955, **33**, 575.
12. Einset, E., and Clark, W. L., *J. Biol. Chem.*, 1958, **231**, 703.
13. Davidson, F. M., and Long, C., *Biochem. J.*, 1958, **69**, 458.

DISCUSSION

DR. J. H. HAMENCE opened the discussion by saying that he imagined every food chemist had had trouble from time to time with the estimation of egg in food products. It was the old story of estimating a small amount of one constituent in the presence of relatively large amounts of inert matter and this particular problem was complicated by the fact that egg products had the awkward habit of breaking down during the preparation of a foodstuff and also during subsequent storage. He congratulated the authors on bringing a new approach to this problem; further, the work carried out by the authors to show

the type of allowance that had to be made for other choline-containing foodstuffs was a valuable addition to our knowledge.   He had not appreciated before that dried milk products, for instance, had such a high natural content of choline.   There was one particular question he would like to ask the authors, namely, what in their opinion was the best method of applying the new technique that they had just described to the estimation of egg in a relatively tough material like egg macaroni or egg noodles.   Was it necessary to give such a preparation a preliminary treatment in order to destroy the bulk of the starchy material.

MR. CASSON and MR. GRIFFIN replied that they had not investigated starchy products in detail, but preliminary tests had indicated that satisfactory recoveries could only be obtained after an initial digestion of the material at 60° to 70° C with a mixture of proteinase and amylase.   For noodles, the amount of choline derived from the flour was at least equal to that derived from the egg, but in two samples containing 1·85 and 4·45 per cent. of whole-egg solids recoveries of 98 and 107 per cent., respectively, had been obtained after the digestion treatment and correction for choline in the flour used (0·087 per cent.).   On a sponge cake and on a dry cake mix recoveries of 97 and 102 per cent., respectively, had been obtained after correction for choline (0·06 per cent.) in the flour.   The extent of variation in the choline content of different types of flour had not yet been investigated.

MR. A. L. BACHARACH asked if an improvement in the authors' elegant technique could be made by taking a tip from the somewhat analogous field of biological assay.   Some increased standardisation might be assured, and possibly considerable waste of precious enzyme solution avoided, by checking each batch either against a sample of some suitable and chemically pure substrate, such as choline phosphate, or by adopting an arbitrary "unit" of activity based on a particular "standard" sample of extract with which subsequent batches could be compared.   It seemed a little optimistic to assume that all batches of cabbage would give rise to enzyme preparations having just about the same amount of activity in the same dry weight of material.

MR. GRIFFIN replied that standardisation of the enzyme preparation would undoubtedly be an improvement that would save enzyme, and in the commercial preparation of the enzyme it would be necessary to do this.   The use of a synthetic substrate, such as choline phosphate, however, might not be satisfactory; there is evidence (Davidson and Long, Biochem. J., 1958, 69, 458) that choline is liberated more slowly from this compound than from phospholipids.   Purified ovolecithin might be more suitable for standardising the enzyme preparation.

MR. E. Q. LAWS suggested that the enzyme activity of the cabbage extract might be influenced by the age of the cabbage from which it was obtained.

MR. GRIFFIN replied that this had not been investigated.   All the cabbages used had been mature-hearted cabbages obtained from commercial sources, and no significant variations had been observed.

MR. P. MORRIES asked to what extent the interference of mustard with the enzymic-hydrolysis method was due to the fact that the enzyme preparation was not pure, but was possibly a mixture of enzymes.

MR. GRIFFIN said that it seemed unlikely that any other enzymes present were responsible for the interference caused by mustard.   Choline determinations had been made on mustard both before and after enzymic hydrolysis, and similar values had been obtained.

MISS M. OLLIVER asked if, under the conditions used, enzymes other than lecithinase might give rise to degradation products, e.g., polygalacturonic acid residues if pectin were present in the foodstuff as emulsion stabiliser, and how far would these interfere.

MR. GRIFFIN replied that polygalacturonic acid residues would pass through the cation-exchange column and then be removed before the choline was eluted.

DR. H. C. LOCKWOOD said that the authors' method was intended for the determination of egg in salad creams, but one slide had shown that soya lecithin contained more than 2 per cent. of choline, which was appreciably more than dried egg.   Lecithin was a powerful emulsifier and could be used in the preparation of salad cream.   Ground-nut or cottonseed lecithin could also be used.   The choline determination would then give a wrong impression of the amount of egg present.   This would restrict the method to the examination of a manufacturer's product in their own laboratories, so as to confirm, or otherwise, that a known recipe was being maintained.

MR. GRIFFIN agreed that soya products cause serious interference both in the choline method and in the phosphorus method.   The ratios of choline to phosphorus pentoxide in soya lecithin and ovolecithin are, however, different (approximately 0·4 and 1·1, respectively) and advantage might perhaps be taken of this difference to decide whether or not soya phospholipids were present.

A Neutral Reagent for the Routine Determination of Fat in Milk and Milk Products

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Two reagent solutions are described for the determination of fat in milk and dairy products. The solutions are (*a*) an alkaline solution that can be used with or without centrifugation and gives results in good agreement with those found by the Gerber method, and (*b*) a neutral non-corrosive solution, the use of which, when compared with standard methods, gave satisfactory results for 306 samples of milk and 186 samples of various dairy products.

After prolonged use, the alkaline solution has been found to attack the butyrometers. The technique of the test with the neutral solution is simple, and its cost compares favourably with that of the Gerber test.

THE most widely used routine tests for the determination of butter fat, the Gerber and the Babcock methods, involve the use of sulphuric acid. It is therefore not surprising that many attempts have been made to replace this unpleasant reagent by something more innocuous. The use of alkaline or neutral reagents has been advocated by workers in Europe, and methods based on synthetic detergents have been developed in America. Not unnaturally, the detergent reagents described by Schain[1,2] and Sager and his co-workers[3,4] were designed for use with the Babcock apparatus, but Godfrain[5] and Adamo[6] have made attempts to adapt them for use with the Gerber apparatus. Such attempts have not been entirely successful, and the methods remain rather unattractive to those accustomed to the Gerber test, as they are time-consuming and require manipulation of the butyrometers in a bath of boiling water as an essential part of the technique. A comparison of results by the Schain and the Sager methods with those of Babcock and Mojonnier has been made by Hoover, Mucha and Harvey.[7]

Some of the alkaline[8,9,10,11,12] or neutral[13,14,15] solutions require apparatus that is not standard equipment for the Gerber test, some require an undue amount of time or manipulation and none appears to be sufficiently reliable for routine use. A further disadvantage of alkaline reagents, which renders such solutions unsuitable for accurate work, became apparent during the course of the work described in this paper.

ALKALINE REAGENTS

An alkaline reagent solution was devised; it had the following composition—

> Sodium hydroxide, 8 per cent. w/v;
> Sodium salicylate, 4 per cent. w/v;
> *n*-Butyl alcohol, 6·25 per cent. v/v;
> Industrial methylated spirit (66 O.P.), 25 per cent. v/v.

n-Butyl alcohol was used in preference to amyl alcohol, since the latter is of uncertain composition and its effect on the fat reading may vary from batch to batch. This solution gave results in good agreement with those by the Gerber method, but had certain defects. When used in a standard butyrometer, 10·6 ml of milk were required instead of the usual volume and the solution could not be used for samples that had been preserved with mercuric chloride or potassium dichromate.

As a result of the experience gained with this solution, a reagent solution of lower alkalinity was evolved for use with the normal 10·94 ml of milk; this solution had the following composition—

> Sodium hydroxide, 3 per cent. w/v;
> Disodium ethylenediaminetetra-acetate, 1 per cent. w/v;
> Sodium chloride, 10 per cent. w/v;
> *n*-Butyl alcohol, 4·85 per cent. v/v;
> Industrial methylated spirit (66 O.P.), 25 per cent. v/v.

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The substitution of disodium ethylenediaminetetra-acetate for sodium salicylate made it possible to use tap-water instead of distilled water to make up the solution, which could then be used for testing samples containing mercuric salts or potassium dichromate.

Milk samples were tested by measuring 10 ml of the reagent solution into a milk butyrometer and adding 10·94 ml of milk. The butyrometer was stoppered, inverted twice and set aside for 10 minutes in a water bath at 65° C. It was then shaken thoroughly, spun in a centrifuge of the type specified in B.S. 696[16] at 1100 r.p.m. for 5 minutes, set aside in the water bath at 65° C for 3 to 5 minutes, and then read.

When this procedure was used, there was satisfactory agreement between the results and those found by the Gerber method for three hundred samples of raw, pasteurised, homogenised and sterilised milk having fat contents between 2·5 and 6·0 per cent. The mean difference between the results for fat content by the two methods was +0·003 per cent., and the frequency distribution of the results was as follows—

Difference from result by the Gerber method, %	+0·20	+0·15	+0·10	+0·05	0	−0·05	−0·10	−0·15	−0·20
Number of results with this difference	2	6	39	74	74	63	32	6	4

By increasing the proportion of n-butyl alcohol, it was possible to dispense with centrifugation. Although the time taken and the manipulation required do not render this procedure suitable for routine work, it could be of considerable use when a centrifuge is not available. The reagent solution had the following composition—

> Sodium hydroxide, 3 per cent. w/v;
> Disodium ethylenediaminetetra-acetate, 1 per cent. w/v;
> Sodium chloride, 5 per cent. w/v;
> n-Butyl alcohol, 8 per cent. v/v;
> Industrial methylated spirit (66 O.P.), 25 per cent. v/v.

Milk samples were tested by measuring 10 ml of the reagent solution into a butyrometer and adding 10·94 ml of milk. The butyrometer was stoppered, inverted twice and set aside for 10 minutes in a water bath at 65° C. It was shaken thoroughly, returned to the water bath for 5 minutes, inverted seven times, returned to the water bath and set aside for 10 minutes with the stopper downwards, after which the percentage of fat was read. The results for one hundred and fifty samples of raw and pasteurised milk having fat contents between 2·5 and 6·0 per cent. were compared with the corresponding results by the Gerber method. The mean difference between the results for fat content by the two methods was −0·055 per cent., and the frequency distribution of the results was as follows—

Difference from result by the Gerber method, %	+0·20	+0·15	+0·10	+0·05	0	−0·05	−0·10	−0·15	−0·20
Number of results with this difference	1	3	5	12	32	31	38	19	9

EFFECT OF ALKALINE REAGENTS ON BUTYROMETERS—

When the work on the first alkaline reagent solution had been completed, the butyrometers were re-calibrated. It was found that the reagent had attacked the glass and caused an increase in the volume of the graduated stem. Of the twenty-six butyrometers used, eleven retained their original limits of error of ±0·02 per cent., twelve now had an error of −0·05 per cent. and three had an error of −0·10 per cent. Six butyrometers were used in a controlled experiment to simulate the effect of five hundred tests, and it was found that one retained its original limits of error of ±0·02 per cent., one had an error of −0·05 per cent. and four of −0·10 per cent. Soda-glass tubing increased in volume by 0·9 per cent. when subjected to similar treatment.

As a result of these findings, the second alkaline reagent solution was examined for corrosive effects. The breakage rates at depot laboratories indicated that the maximum life of a butyrometer was equivalent to one thousand tests, and an experiment was carried out to simulate the effect of this number of tests. Four butyrometers from each of three suppliers, four Pyrex-glass butyrometers and soda-glass and Pyrex-glass tubing were used. The twelve ordinary butyrometers decreased in accuracy to an average error of −0·14 per cent. and the Pyrex-glass butyrometers to an average of −0·11 per cent. Both soda-glass and Pyrex-glass tubing increased in volume by 1·2 per cent.

These results indicate that the use of alkaline reagent solutions causes a progressive decrease in the accuracy of a butyrometer and makes it unsuitable for accurate work within the period of its expected life. The use of any reagent containing 3 per cent. or more of sodium hydroxide cannot be recommended, and this rules out many of the published reagents. A surprising feature of the reagent attack was that Pyrex glass proved to be only slightly more resistant than soda glass.

NEUTRAL REAGENTS

Initial experiments showed that the most promising solution for dissolving the solids-not-fat of milk was a mixture of trisodium citrate, sodium salicylate and disodium ethylene-diaminetetra-acetate. Although the mixture was efficient for the dissolution, it failed to break down the fat emulsion completely, and accurate readings could not be obtained. This difficulty was overcome by the use of a polyoxyethylene derivative of sorbitol trioleate (Tween 85) in conjunction with ethanol; this combination gave a sharp separation between the fat column and the aqueous layer. At this stage, a comparison of readings obtained with use of this reagent solution indicated that they were slightly lower than those by the Gerber method; this difference was removed by the addition of *n*-butyl alcohol to the solution.

The reagent solution in its final form is an opalescent liquid that slowly separates into two clear layers, which re-combine instantaneously after one inversion of the containing vessel. The mixture has a pH of 7·8. It does not attack glass when used for testing milk, and butyrometers subjected to conditions that simulated the effect of one thousand tests did not suffer any alteration in volume or any decrease in accuracy.

METHOD

APPARATUS—

Use the apparatus specified[16] for determining fat in milk and milk products by the Gerber method.

PREPARATION OF REAGENT SOLUTION—

The reagent solution has the following composition—

Trisodium citrate, 5 per cent. w/v;
Sodium salicylate, 5 per cent. w/v;
Disodium ethylenediaminetetra-acetate, 1 per cent. w/v;
Tween 85, 1·1 per cent. w/v;
n-Butyl alcohol, 3 per cent. v/v;
Industrial methylated spirit (66 O.P.), 25 per cent. v/v.

Weight the Tween (obtainable from Honeywill - Atlas, Devonshire House, Mayfair Place, Piccadilly, London, W.1) into a small beaker, dissolve it in part of the methylated spirit, transfer to a calibrated vessel, and use the remainder of the spirit to rinse the beaker. Add the *n*-butyl alcohol to the mixture. Dissolve the disodium ethylenediaminetetra-acetate in hot water, and neutralise the solution to phenolphthalein with N sodium hydroxide. Dissolve the citrate and salicylate in this mixture, and then add it to the mixture in the calibrated vessel. Cool to 20° C, dilute to the mark with water, and mix.

When the solution has been set aside for some hours, a thin layer of liquid separates at the top of the reagent. Just before use, invert the container once or twice to disperse this layer.

PROCEDURE FOR RAW, PASTEURISED, HOMOGENISED OR STERILISED MILK—

Measure 10 ml of reagent solution into a milk butyrometer, and add 10·94 ml of well mixed milk by pipette. Close the butyrometer with a stopper, invert twice, and place in a water bath at 65° C for 5 minutes. Shake the butyrometer until the contents are thoroughly mixed, and invert it two or three times while shaking. Spin the butyrometer in a centrifuge at 1100 r.p.m. for 5 minutes immediately after mixing. Place the butyrometer, stopper downwards, in the water bath at 65° C for at least 3 minutes. Read the percentage of fat in the usual manner.

PROCEDURE FOR PLAIN AND FLAVOURED YOGHOURT—

Weigh 11·3 ± 0·05 g of sample into a small dish, and transfer to a milk butyrometer by pouring it down a glass rod. Use 10 ml of reagent solution in two portions to rinse the remainder of the sample into the butyrometer. Complete the test as for milk.

Fat content, per cent. = Butyrometer reading + 0·10.

PROCEDURE FOR CREAM—

Weigh 1 ± 0.002 g of sample into a small dish. Transfer to a milk butyrometer containing 10 ml of reagent solution, and rinse the remainder of the sample into the butyrometer with sufficient warm water to bring the level of the contents to the shoulder below the neck. Complete the test as for milk.

Fat content, per cent. = Butyrometer reading × 11·1.

PROCEDURE FOR CHEESE AND CHEESE SPREAD—

Hard cheese—Weigh 3 ± 0.01 g of grated sample into a stoppered funnel. Transfer to a cheese butyrometer containing 10 ml of reagent solution, and add water to bring the level of the contents to the shoulder below the neck.

Soft cheese, etc.—Weigh 3 ± 0.01 g of sample into a small dish. Add 4 ml of hot water, and stir with a short glass rod to disperse the sample. Transfer to a cheese butyrometer containing 10 ml of reagent solution. Rinse the dish with two 2·5-ml portions of hot water, and add the rinsings to the solution in the butyrometer.

Complete the test as for milk (shake the mixture until all particles of curd have dissolved), and read the percentage of fat in the usual manner.

PROCEDURE FOR CONDENSED OR EVAPORATED MILK—.

Weigh 4 ± 0.01 g of sample into a small dish. Add 3 ml of hot water, and stir to disperse the sample. Transfer to a milk butyrometer containing 10 ml of reagent solution, and rinse the dish with two 2·5-ml portions of hot water. Add the rinsings to the solution in the butyrometer, insert the stopper, invert twice, and place in a water bath at 65° C for 5 minutes. Shake vigorously, and replace in the water bath for 5 minutes. Shake the butyrometer again, spin in a centrifuge at 1100 r.p.m. for 5 minutes, replace in the water bath at 65° C for 5 minutes, and then read.

Fat content, per cent. = Butyrometer reading × 2·87.

PROCEDURE FOR DRIED MILK—

Weigh 1.69 ± 0.01 g of sample into a small dish. Add 5 ml of hot water, and stir with a short glass rod to disperse the sample. Transfer to a milk butyrometer containing 10 ml of reagent solution, and rinse the dish with two 2·5-ml portions of hot water. Add the rinsings to the solution in the butyrometer, and complete the test as for milk (shake the mixture until no solid particles are visible.)

Fat content, per cent. = Butyrometer reading × 6·72.

PROCEDURE FOR ICE-CREAM—

Treat 4 ± 0.01 g of sample exactly as for condensed milk.

NOTE—Because of the variety of fats and emulsifiers used by different manufacturers of ice-cream, the factor for converting butyrometer reading to fat content varies somewhat with the source of the product. Once the factor has been determined for any particular variety of ice-cream, the procedure can be used for routine control purposes. Although some varieties of ice-cream contain material that is insoluble in the reagent solution, its presence does not interfere with the reading.

PROCEDURE FOR ICE-CREAM POWDERS AND OTHER DRIED PRODUCTS CONTAINING SUBSTITUTED
        FATS—

Treat 1.69 ± 0.01 g of sample exactly as for dried milk.

NOTE—The factor for converting butyrometer reading to fat content varies with the nature of the fat, but can be used for the routine control of any product when the ratio has been established.

RESULTS

Fat contents found by the proposed method were compared with those found by established methods. The Gerber method[17] was used as a reference for milk, cream and cheese, and the appropriate gravimetric procedures were used for condensed milk,[18] dried milk[19] and ice-cream.[20] The results are shown in Tables I and II.

For condensed milk, dried milk and ice-cream, factors for converting scale readings to percentage of fat were established by comparing the results found by the two procedures.

Fourteen samples of ice-cream, containing 9·89 to 13·18 per cent. of fat, from four manufacturers were tested; the results were as follows—

Manufacturer			A	B	C	D
No. of samples tested			7	4	2	1
Conversion factor	..	..	2·88	2·77	2·86	2·87

Eight samples of powders with substituted fat (fat content between 27·30 and 31·60 per cent.) were tested.   The conversion factor for four ice-cream powders varied from 6·82 to 6·92; for the remaining four powders, which were substituted with various fats, the conversion factor varied from 6·59 to 6·91.

TABLE I

FREQUENCY DISTRIBUTION OF DIFFERENCE BETWEEN RESULTS BY PROPOSED AND GERBER METHODS FOR MILK

The fat contents of the milk samples were between 2·5 and 6·2 per cent.

Type of milk	No. of samples tested	No. of results with the following differences from the Gerber result—					Mean difference in fat content, %
		+0·10%	+0·05%	0	−0·05%	−0·10%	
Raw and pasteurised.. ..	210	3	71	111	24	1	+0·012
Homogenised and sterilised ..	96	0	13	32	49	2	−0·021
Total	306	3	84	143	73	3	+0·002

TABLE II

FREQUENCY DISTRIBUTION OF DIFFERENCE BETWEEN RESULTS BY PROPOSED AND REFERENCE METHODS FOR VARIOUS MILK PRODUCTS

No. of samples tested	Range of fat content, %	Difference between fat contents by proposed and reference methods, %	No. of results with this difference	Mean difference in fat content, %
Plain and flavoured yoghourt—				
48	2·90 to 3·45	+0·10 +0·05 0 −0·05 −0·10	0 12 23 13 0	−0·001
Cream—				
48	17 to 51	+1·0 +0·5 0 −0·5 −1·0	0 9 36 3 0	+0·06
Cheese—				
24*	21·0 to 41·0	+0·6 +0·3 0 −0·3 −0·6	0 7 9 8 0	−0·01
Condensed milk—				
25	7·95 to 10·52	+0·16 to +0·20 +0·06 to +0·15 0 to 0·05 −0·06 to −0·15 −0·16 to −0·20	2 7 8 7 1	+0·014
Dried milk—				
17	26·34 to 27·30	+0·21 to +0·40 0 to 0·20 −0·21 to −0·40	3 10 4	+0·01

* Eleven samples of Cheddar, one sample each of Caerphilly, Double Gloucester, Cheshire, Edam, Parmesan, Gruyère, Provelone, Danish Blue and Gorgonzola and four samples of cheese spreads.

DISCUSSION OF THE METHOD

NEUTRAL REAGENT—

The reagent solution is best prepared in the manner described. It was found to be more convenient to use disodium ethylenediaminetetra-acetate and to convert it to the trisodium salt *in situ* rather than to use the latter material, which is hygroscopic. Tap-water can be used.

From its method of preparation, it seemed possible that there might be some variation of properties between different batches of Tween 85. Examination of samples from one manufacturer showed that batches made in England, Germany and America possessed suitable properties and that the associated companies had achieved considerable uniformity of composition. A sample from another manufacturer was unsuitable, and analysis showed that the proportions of sorbitol, oleic acid and polyoxyethylene oxide in this product were different from those in the Atlas materials.

In practice, the suitability of any sample of Tween can be tested by incorporating it in a trial batch of the reagent and noting whether or not it gives a clean separation of fat and a correct reading when used with milk of known fat content.

ANALYTICAL PROCEDURE—

As with all reagents other than strong acid, no increase in temperature takes place when the reagent solution is mixed with milk, and an initial period of heating is necessary. Some compensation for this extra time is gained because the reagent solution is complete in itself and does not have to be placed in the butyrometer in two parts, as, for example, do sulphuric acid and amyl alcohol; for homogenised products, the proposed method is more rapid than the Gerber test, as only one centrifugation is necessary. The time of heating is not critical, provided that not less than 5 minutes are allowed for the mixture to attain a temperature of 60° to 65° C. No adverse effect is produced if the butyrometer is set aside in the water bath for periods up to 7 hours either before or after centrifugation. In such circumstances, a third layer appears between the fat and the aqueous layers, but the volume of the fat layer is unaltered.

PRESERVED SAMPLES—

The reagent solution is unaffected by the presence of formalin, mercuric chloride or potassium dichromate.

SWEETENED PRODUCTS—

The reagent solution is particularly suitable for materials containing sucrose, as no charring or discolouration is produced.

SEPARATED MILK—

In its present form, the reagent solution is not suitable for testing separated milk. For milks having fat contents from 1 to 2 per cent., an approximate result can be obtained by adding a correction of 0·25 per cent. to the observed reading.

COST—

The present cost of reagents for the Gerber test is 0·32 pence per test. The estimated cost of the constituents of the neutral reagent solution, bought on the same scale as those for the Gerber test, is 0·27 pence per test.

CONCLUSIONS

It has been shown that it is possible to replace the sulphuric acid, as used in the Gerber test, by an alkaline reagent solution and to obtain satisfactory results, even without centrifugation. Although this method may prove to be of value in certain circumstances, it would not be suitable for routine use, as the reagent solution attacks the butyrometers in the course of time and so destroys their accuracy.

A neutral reagent solution has been devised and found to be non-corrosive to glassware. This solution can be used with standard Gerber apparatus to determine the fat content of milk, cream, yoghourt, cheese, condensed milk, ice-cream and dried milk with an accuracy equal to that of the corresponding Gerber method, It possesses certain advantages over acid in that it is non-corrosive to centrifuges, baths, stands, clothing and skin; it is particu-

larly suitable for sweetened products, such as condensed milk or ice-cream, as no decomposition or charring of sugars takes place, and homogenised products require only one centrifugation. As the solution is neutral, there is no tendency for fat hydrolysis to take place if milk and the reagent solution remain in contact for prolonged periods of time.

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REFERENCES

1. Schain, P., *Science*, 1949, **110**, 121.
2. ——, *Milk Dealer*, 1950, **40**, 49.
3. Sager, O., and Sanders, G. P., *Milk Ind. Found. Conv. Proc.*, 1952, 4, 29.
4. Sager, O., Sanders, G. P., Norman, G. H., and Middleton, M. B., *J. Ass. Off. Agric. Chem.*, 1955, **38**, 931.
5. Godfrain, J. C., *Rev. Med. Vet.*, 1955, **106**, 370.
6. Adamo, G., *Boll. Lab. Chim. Prov.*, 1952, 4, 95; *Dairy Sci. Abstr.*, 1954, **16**, 416.
7. Hoover, S. R., Mucha, T. J., and Harvey, W. R., *J. Dairy Sci.*, 1958, **41**, 398.
8. Hoyberg, H. M., U.S. Patent 1,521,953 (1925).
9. Dimov, N., *Bull. Inst. Anim. Husb. Bulgarian Acad. Sci.*, 1952, 3 and 4; *J. Dairy Sci.*, 1954, **37** 103A.
10. Houston, J., *J. Soc. Dairy Tech.*, 1955, 8, 47.
11. Effern, J., *Milchwissenschaft*, 1952, **7**, 231; *Dairy Sci. Abstr.*, 1954, **16**, 238.
12. Iannucci, M., *Acta Med. Vet.*, 1955, **1**, 253; *Dairy Sci. Abstr.*, 1957, **19**, 425.
13. Gentilini, L., *Latte*, 1950, 24, 219; *Dairy Sci. Abstr.*, 1952, **14**, 373.
14. Salerno, A., *Latte*, 1954, **28**, 275; *Dairy Sci. Abstr.*, 1955, **17**, 165.
15. Ferrara, B., and de Paolis, P., *Le Lait*, 1957, **37**, 481.
16. British Standard 696 : Part 1 : 1955.
17. British Standard 696 : Part 2 : 1955.
18. British Standard 1742 : 1951.
19. British Standard 1743 : 1951.
20. British Standard 2472 : 1954.

The Microbiological Detection of Preservatives other than Benzoic or Sorbic Acid in Margarine with a Sodium Chloride-tolerant Yeast as Test Strain

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Because none of the test strains used with success for detecting preservatives in other foods can be used for the examination of the aqueous phase of margarine, which may contain 6 per cent. of sodium chloride, some sodium chloride-tolerant micro-organisms were isolated and tested for sensitivity to antimicrobial agents. A strain of *Candida brumptii* isolated from surface water was found to be sufficiently sensitive.

The margarine or heavily salted butter is heated to $43° \pm 1°$ C to yield a serum, which is enriched with dextrose and yeast extract, standardised to a sodium chloride concentration of 9 per cent. and pH 7, filtered sterile, solidified with agar and inoculated with between 10^2 and 10^3 cells of test strain per ml. A 3-ml portion of inoculated agar is incubated, as a strip, for 48 hours at 30° C. Growth inhibition is considered to be significant when there develop 10 per cent. or less of the number of colonies found in a blank test.

The method permits the detection of 5×10^{-3} per cent. of sodium methyl-*p*-hydroxybenzoate, 5×10^{-5} per cent. of ethyl monobromoacetate and 2×10^{-3} per cent. of chlorhexidine, tetramethylthiuramdisulphide or γ-C_{12}-lactone. Under the conditions of the test, benzoic acid and sorbic acid, preservatives permitted in some countries, do not interfere.

THE addition of preservatives, except benzoic acid and possibly sorbic acid,[1] to margarine is prohibited in practically all countries. However, incorporation of either of these preservatives

with margarines in which the pH of the aqueous phase (serum) is about 6 or more is ineffective, as at this pH these acidic inhibitory substances lose most of their antimicrobial activity.[2] Some manufacturers, therefore, occasionally add small amounts of other powerful antimicrobial agents, which are often highly toxic to the human consumer.[3]

For the detection of such preservatives, chemical and physico-chemical methods can be used with some success. However, even with modern methods of detection, such as partition chromatography and infra-red spectroscopy, the search for an unknown antimicrobial agent may be complicated and time-consuming, because some way of concentrating the unknown substance must always precede the final identification, and this part of the procedure may present difficulties. Identification may sometimes be impossible, *e.g.*, when the substance used has been prepared by, or on behalf of, a food producer and has a virtually unknown structure.

Non-specific microbiological methods for determining the presence of antimicrobial agents in general are thus still useful in food analysis. So far, variants of the Kluyver fermentation test have often been used.[3] Unfortunately, this method is ineffective for margarine, as the aqueous phase—in which microbial growth may occur and with which the test is therefore carried out—is always inhibitory to the usual test organism, *Saccharomyces cerevisiae* (baker's yeast), which can only tolerate 0·5 per cent. of sodium chloride, whereas margarine serum may contain at least 5 per cent. of sodium chloride.

If the serum were sufficiently diluted before inoculation, this difficulty could be overcome, but the sensitivity of the test would be greatly reduced. This paper describes the development of a microbiological test for use with sera containing 5 to 9 per cent. of sodium chloride.

EXPERIMENTAL

PRELIMINARY RESEARCH—

Tests with osmophilic yeasts of the type *Saccharomyces rouxii*[4],[5] revealed that these organisms were extremely sensitive to benzoic acid, even when the pH had been deliberately increased to inactivate most of the preservative. Trials with *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*[6] and other sodium chloride-tolerant bacteria isolated from river water showed that these organisms were insufficiently sensitive to preservatives. Eventually, a yeast strain isolated from river water by enrichment in 6 per cent. sodium chloride - dextrose - yeast extract broth of pH 3·5 and identified as *Candida brumptii* proved to be sufficiently sensitive. As this strain was only weakly saccharolytic, it was necessary to use impaired growth as the measure of inhibition. Because tests in liquid media appeared to be unreliable, owing to contamination by other sodium chloride-resistant organisms (especially bacilli), a solidified medium prepared from the margarine serum was used.

DEVELOPMENT OF THE METHOD—

Because the yield when the serum is rendered from margarine is often low,[7] it was desirable to use as little serum as possible. This suggested the use of Stirling, Stevens and Lawley's agar-strip technique,[8] in which 0·1 ml of a suspension of the test organism is mixed with about 3 ml of agar in a culture tube and the inoculated agar is allowed to set with the tube held almost horizontal, so that a strip about 4 inches long is formed.

It was necessary to standardise both the nutritional and the inhibitive potential of the medium. To accomplish the former, it was decided to use superoptimal amounts of yeast extract and dextrose. As the test was intended to indicate the presence of illegal preservatives only, any benzoic acid or sorbic acid present had to be inactivated by adjusting the pH of the medium to about 7. Finally, the sodium chloride content of the test medium was standardised to 6 per cent., because, owing to the dilution of the serum involved in preparing the test medium (*vide infra*), this level corresponds to about 9 per cent. of sodium chloride in the aqueous phase of the margarine, a level rarely exceeded in Dutch produce.

To render more easily visible the colonies growing in the often opaque serum agar, 15 mg per litre of bromocresol purple were added to the medium. However, the colour change of this indicator occurs later than visible growth and is therefore not used as a criterion.

METHOD

PREPARATION, ANALYSIS AND STANDARDISATION OF SERUM—

Transfer about 125 g of the sample to a plugged sterile 500-ml Erlenmeyer flask having a neck about 5 cm in diameter. Heat in a thermostatically controlled water bath between

42° and 44° C until at least 5 ml of serum have been rendered; this generally takes less than 2 hours. If sufficient serum is not obtained in this way, spin the mixture in a centrifuge, free the lower layer, which is often a milky paste, from much of the fat by careful decantation, and mix it with an equal volume of warm (about 50° C) sterile 0·1 M phosphate buffer solution of pH 8. Spin the mixture in a centrifuge for 30 minutes at 2000 r.p.m. and 15·5-cm radius.[7]

Determine the approximate sodium chloride content by argentimetric titration of the chloride ion. If the sodium chloride content is not 9 per cent., add either water or sterile analytical-reagent grade sodium chloride to bring the sodium chloride content to 9·0 ± 0·2 per cent.

ENRICHMENT AND ADJUSTMENT OF SERUM—

To a 10-ml portion of serum add 0·2 ml of a sterile 25 per cent. w/v solution of dehydrated yeast extract, 0·2 ml of sterile 50 per cent. w/v pure dextrose solution and 0·1 ml of a sterile 0·15 per cent. w/v bromocresol purple solution.

Adjust the pH of the serum to 6·9 ± 0·1 by adding a few drops of a sterile 30 per cent. w/v solution of analytical-reagent grade potassium hydroxide. Filter the adjusted serum through a G5 sintered-glass filter.

INOCULUM—

From a 1 per cent. dextrose - 0·5 per cent. yeast extract - 6 per cent. sodium chloride - agar culture of *C. brumptii*, prepare a subculture in 1 per cent. dextrose - 0·5 per cent. yeast extract - 6 per cent. sodium chloride - water by incubation for about 24 hours at 30° C. Store this culture in a refrigerator until required, and count the number of viable cells present per millilitre, *e.g.*, by using poured plates of malt agar or 2 per cent. dextrose - 0·5 per cent. yeast extract - agar of pH 3·5 ± 0·1.[9]

PREPARATION OF INOCULATED STRIPS—

Mix 0·1 ml of a dilution of the culture, containing between 3×10^3 and 10^4 viable cells per ml, 2 ml of adjusted and sterilised serum and 1 ml of a sterile 4·5 per cent. w/v aqueous solution of agar in a sterile culture tube. With the tube in an almost horizontal position, allow the strips to solidify for about 1 hour at about 15° C. Prepare two such tubes.

INCUBATION AND INTERPRETATION—

Incubate the inoculated strips for 48 hours at 30° C. Use as a blank two similarly inoculated strips of pH 6·9 ± 0·1 containing 6 per cent. of sodium chloride, 1 per cent. of dextrose, 0·5 per cent. of yeast extract, 0·3 per cent. of sodium benzoate, 15 mg per litre of bromocresol purple and 1·5 per cent. of agar. Determine the numbers of colonies in the four tubes.

The coefficient of variation of a count of *C. brumptii* in sodium chloride agar, calculated from 55 experiments, appeared to be 18 per cent. or less (average 11 per cent.). This means that 80 per cent. inhibition is significant. Growth is considered to have been inhibited when the number of colonies in a sample strip is 10 per cent. or less of the logarithmic average number of colonies in the blank strips.

DISCUSSION OF RESULTS

The levels of detection in a solidified artificial margarine serum containing—as well as dextrose, dehydrated yeast extract, bromocresol purple and agar—6 per cent. of sodium chloride and 0·3 per cent. of sodium benzoate were determined for a few old established as well as more recently developed antimicrobial agents. Sodium methyl-*p*-hydroxybenzoate, ethyl monobromoacetate, the diacetate of bis(*p*-chlorophenyldiguanido)hexane (chlorhexidine),[10,11] tetramethylthiuramdisulphide (TMTD) and γ-C$_{12}$-lactone were studied.

TMTD was chosen because it is effective as an antioxidant in butter[12] and is also a powerful fungistatic agent.[13] A lactone-type compound was studied because δ- and ε-lactones have been suggested as flavouring substances in margarine[14,15] and it is known that β-lactones are powerful antimicrobial agents.[16] TMTD and γ-C$_{12}$-lactone were dissolved in pure ethanol, each solution having a concentration such that the amounts of ethanol in the agar strips did not exceed 0·5 per cent. v/v, a level apparently non-inhibitory to *C. brumptii* under the conditions of the test.

The results are shown in Table I, in which all levels represent the logarithmic average of at least three experiments. It can be seen that sorbic acid does not interfere with the test at a concentration of 0·3 per cent.

TABLE I

LEVELS OF DETECTION OF SOME ANTIMICROBIAL AGENTS

Compound	Level of detection.* %
Sodium methyl-p-hydroxybenzoate ..	5×10^{-2}
Ethyl monobromoacetate	5×10^{-5}
TMTD	2×10^{-3}
Chlorhexidine..	2×10^{-3}
γ-C_{15}-Lactone	2×10^{-3}
Sorbic acid	$\geqslant 3 \times 10^{-1}$

* The concentration at which the number of colonies seeded
is reduced to 10 per cent. or less.

The γ-lactone studied appeared to possess fungistatic properties of the same potency as that of TMTD or chlorhexidine.

It may be reasoned that another modern preservative, viz., 3-acetyl-6-methylpyran-2:4-dione (dehydracetic acid), cannot be detected in this way because its antimicrobial action is considerably reduced at a pH of about 7.[17,18] Although this cannot be denied, it should not be forgotten that this preservative, in practice, is only faintly active at higher pH values, so that there are better preservatives for use in margarines having neutral sera. On the other hand, products having an acid aqueous phase can be no better protected against microbial spoilage than by benzoic or sorbic acid,[1] so that there is no reason for a manufacturer to risk prosecution by using dehydracetic acid in those products.

By contrast, the preservatives studied in this investigation could profitably be used in products of almost neutral pH, for which both benzoic and sorbic acid cannot be used.[2]

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REFERENCES

1. Becker, E., and Roeder, I., Fette, Seif. Anstrichmitt., 1957, 59, 321.
2. von Schelhorn, M., Dtsch. Lebensmitt. Rdsch., 1954, 50, 267.
3. Mossel, D. A. A., Food Manuf., 1956, 31, 190.
4. Bernaerts, M. J., Z. LebensmittUntersuch., 1956, 104, 405.
5. Mossel, D. A. A., Antonie van Leeuwenhoek, 1951, 17, 146.
6. Baetsle, R., Mossel, D. A. A., and Verheyden, H., Ann. Falsif., 1955, 48, 412.
7. Mossel, D. A. A., and Zwart, H., Netherlands Milk & Dairy J., 1958, 12, 218.
8. Stirling, A. C., Stevens, M. K., and Lawley, D. N., J. Gen. Microbiol., 1950, 4, 339.
9. "Standard Methods for the Examination of Dairy Products," Tenth Edition, American Public Health Association and Association of Official Agricultural Chemists, New York, 1953.
10. Davies, G. E., Francis, J., Martin, A. R., Rose, F. L., and Swain, G., Brit. J. Pharmacol. Chemother., 1954, 9, 192.
11. Beeuwkes, H., Antonie van Leeuwenhoek, 1958, 24, 49.
12. Tollenaar, F. D., Netherlands Milk & Dairy J., 1951, 5, 46.
13. Manten, A., Klöpping, H. L., and van der Kerk, G. J. M., Antonie van Leeuwenhoek, 1950, 16, 45.
14. Boldingh, J., Begemann, P. H., Lardelli, G., Taylor, R. J., and Weller, W. T., Brit. Pat. 748,661 (August 5th, 1953).
15. Taylor, R. J., and Weller, W. T., Brit. Pat. 748,801 (August 5th, 1953).
16. Hartman, F. W., and Lo Grippo, G. A., J. Amer. Med. Ass., 1957, 164, 258.
17. Mossel, D. A. A., and de Bruin, A. S., Antonie van Leeuwenhoek, 1954, 20, 233.
18. Mossel, D. A. A., and Tollenaar, F. D., Ibid., 1955, 21, 247.

The Complete Analysis of Tocopherol Mixtures

Part I. Introduction and an Investigation into Differential Coupling Reactions

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An outstanding problem in the field of vitamin-E analysis is the separation of β- from γ-tocopherol and ϵ- from η-tocopherol. Attempts have been made to unite methods based on differential coupling reactions with paper-chromatographic analysis in order to achieve this separation. Two coupling reactions have been studied. The reactions of the four tocopherols with diazotised o-dianisidine have been investigated fully, and earlier results with this reagent have been explained. A new coupling reagent for the tocopherols, 2 : 6-dichloro-p-benzoquinone-4-chloroimine, has been studied. It has been shown that β- and ϵ-tocopherol undergo oxidation in the presence of the two reagents and cannot be recovered quantitatively enough to allow the use of these coupling reactions in an analytical procedure.

SEVEN tocopherols have now been isolated from natural sources. Each may contribute towards the vitamin-E activity of vegetable oils, and, as they differ considerably in biological activity, their individual determinations are of some importance. Two main problems exist. First, the tocopherols, as a group, must be separated from concomitant phenolic, pseudo-phenolic and other reducing substances; secondly, the individual members of the group (of which any or all may be present) must be separated from each other. The former problem has been almost entirely solved by the introduction of reversed-phase paper chromatography[1,2]; a partial solution to the latter has been provided by two-dimensional paper-chromatographic analysis.[3] Two-dimensional chromatography separates the seven tocopherols from all other interfering materials so far encountered and presents them as five chromatographic zones. From these zones, the tocopherols (or their mixtures) can be removed and then determined. Three of the five zones contain α-, ζ- and δ-tocopherol and the remaining two zones individually contain inseparable mixtures of β- and γ-tocopherol and ϵ- and η-tocopherol. The separation of these two mixtures is still an outstanding problem in the field of vitamin-E analysis and is considered in this paper and in Part II of this series.[4]

Before the discovery of ϵ-, δ- and η-tocopherol, three methods had been proposed for the determination of certain individual tocopherols. Quaife[5] found that β-, γ- and δ-tocopherol gave yellow nitroso-derivatives, which could be separated on a zinc carbonate - Celite column and then estimated colorimetrically at 410 mμ. The method is limited in practice by the low extinction coefficients of the nitroso-tocopherols and can in general be applied only to relatively high-potency products. Quaife's chromatographic separation, in practice, would be complicated by the presence of ϵ- and η-tocopherol, whose nitroso-derivatives are inseparable on zinc carbonate columns from those of γ- and β-tocopherol.

Baxter, Robeson, Taylor and Lehman[6] proposed to determine mixtures of α-, β- and γ-tocopherol by a method based on their different rates of oxidation by silver nitrate under carefully controlled conditions. The three tocopherols gave red o-quinones and, from calibration curves, the percentage of each tocopherol in a mixture could be calculated from the intensity of colour produced. The full details of the method do not appear to have been published, and it is doubtful whether the procedure is a useful one.

The most widely used method for differentiating between individual tocopherols, due to Weisler, Robeson, and Baxter,[7] is based on an earlier discovery by Quaife,[8] who found that γ-tocopherol coupled with diazotised p-nitroaniline to give a red dye, whereas β-tocopherol did not. Weisler *et al.* substituted the much more stable diazotised o-dianisidine reagent. This reagent gives, with γ- and δ-tocopherol, red dyes whose intensities depend on the pH of the reaction medium. By carrying out the reaction under two sets of conditions, Weisler *et al.* were able to distinguish between γ- and δ-tocopherol. Harris, Quaife and Swanson[9] used the dianisidine method to estimate non-α-tocopherols (and hence α-tocopherol by difference) in a large range of foodstuffs. The theoretical basis of the method is, however,

invalidated by the presence of η-tocopherol, which couples, and β-, ϵ- and ζ-tocopherol, which do not.

The dianisidine method itself is known to suffer from some other drawbacks.[10] The coupling is inhibited by the presence of small amounts of fat and even by large amounts of α-tocopherol.

Nevertheless, the reaction is easily carried out and provides a rapid method of distinguishing between β- and γ-tocopherol. When paper chromatography is used for the primary separation, many of the drawbacks would no longer seem to apply, and it was thought that, provided the coupling reaction was quantitative, it could serve as a basis for a new procedure.

It was a practical aim of our investigation to devise a method of measuring β-tocopherol directly; this tocopherol has considerable biological activity, and its accurate measurement in small amounts is desirable. The dianisidine assay method has therefore been re-investigated in detail. In addition, the use of a new coupling reagent for tocopherols, 2:6-dichloro-p-benzoquinone-4-chloroimine (Gibbs' reagent) has been studied. In both reactions, the fate of the uncoupled tocopherol has received particular attention.

MATERIALS AND GENERAL ANALYTICAL METHODS

The α-, β-, ζ- and η-tocopherol were pure synthetic DL specimens. The γ-, ϵ- and δ-tocopherol were pure specimens from natural sources. General methods of tocopherol analysis have been previously described[3] and more recent modifications have been described by Green.[11] Briefly, the chromatographic separation consists of (i) an adsorption run on paper impregnated with zinc carbonate, and (ii) a reversed-phase partition run on paper impregnated with liquid paraffin. The runs can be combined as a two-dimensional separation. In the work described below, which deals mainly with simple separation of known mixtures, only one dimension was generally used and is specified as occasion demands.

THE DIANISIDINE REACTION

Weisler et al.[7] dissolved 50 to 100 μg of γ- and δ-tocopherol in ethanol, brought the solution to a standard pH by adding potassium hydroxide or sodium carbonate solutions, added the reagent and, after 2 or 5 minutes, respectively, extracted the dyes with light petroleum for measurement at 510 mμ. Lehman[10] has recently increased the amount to 200 μg of total tocopherols. Weisler et al. found that the dye prepared from γ-tocopherol in potassium hydroxide had "$E_{1\,cm}^{1\%} = 196$" and from the carbonate reaction "$E_{1\,cm}^{1\%} = 183$." However, the δ-tocopherol dye from the potassium hydroxide reaction had "$E_{1\,cm}^{1\%} = 129$," whereas from the carbonate reaction it had "$E_{1\,cm}^{1\%} = 301$." (The values are placed in quotation marks, since they-are, in effect, arbitrary measurements based on the original weights of tocopherol used for the reactions; neither the structures nor the molecular weights of the dyes were identified.)

GENERAL REACTION METHOD—

The two sets of reaction conditions described by Weisler et al. were observed, except that the amounts of tocopherols used were varied over a wider range. At the end of each reaction, the solution was diluted with 10 ml of water and extracted twice with 10 ml of light petroleum (boiling range 80° to 100° C). The combined solution in light petroleum was washed once with water and then evaporated to dryness under reduced pressure. The residue was dissolved in 0.5 to 1.0 ml of benzene and added, totally or partly, to the starting line of the chromatographic paper.

γ-TOCOPHEROL—

γ-Tocopherol was allowed to react under both conditions of pH. The dyes formed in the two reactions behaved identically when chromatographed; they moved readily on paper impregnated with zinc carbonate, with a benzene - cyclohexane mixture $(1 + 2)$ as mobile phase, and took up a position midway between the positions normally occupied by α- and γ-tocopherol. The dyes did not migrate on reversed-phase chromatography. Even small amounts of unreacted γ-tocopherol could be easily separated from the products of coupling, either by adsorption or by reversed-phase chromatography.

Table I shows the results obtained from a series of reactions. It was found that 200 μg or less of γ-tocopherol reacted nearly quantitatively with the diazo solution under either set of conditions. However, attempts to reproduce the extinction values obtained by Weisler et

TABLE I

RECOVERY OF TOCOPHEROLS AFTER DIAZOTISED DIANISIDINE COUPLING REACTIONS

	Amount of tocopherol taken, μg	Alkali	Reducing agent	Recovery, %
γ-Tocopherol	104	KOH	Ascorbic acid	8
	104	Na$_2$CO$_3$	Ascorbic acid	0
	224	KOH	None	0
	224	Na$_2$CO$_3$	None	0
	500	KOH	None	11
	500	KOH	Ascorbic acid	29
	500	Na$_2$CO$_3$	None	0
	500	Na$_2$CO$_3$	Ascorbic acid	6
η-Tocopherol	100	KOH	None	0
	300	KOH	None	20
	100	Na$_2$CO$_3$	None	0, 4
	300	Na$_2$CO$_3$	None	9
	250	Na$_2$CO$_3$	Ascorbic acid	5
δ-Tocopherol	100	KOH	None	17
	100	KOH	Ascorbic acid	30
	100	Na$_2$CO$_3$	None	0
	100	Na$_2$CO$_3$	Ascorbic acid	0
	400	KOH	None	22
	400	Na$_2$CO$_3$	None	0, 7
	500	Na$_2$CO$_3$	Ascorbic acid	11
β-Tocopherol	112	KOH	None	13
	112	KOH	Ascorbic acid	50
	112	Na$_2$CO$_3$	None	0
	112	Na$_2$CO$_3$	Ascorbic acid	0
	505	KOH	None	39 to 65*

* Recovery range from 4 tests.

al. were not successful. The extinction of the dye varied from reaction to reaction, and, whether from the potassium hydroxide reaction or the sodium carbonate reaction, extinctions never reached more than 75 per cent. of the "$E_{1\,cm}^{1\%}$ values" found by these workers. When larger amounts of γ-tocopherol were used, reaction in potassium hydroxide was not quantitative; from 562 μg, 9 per cent. of unchanged tocopherol was recovered. In sodium carbonate, however, substantially all this amount of γ-tocopherol reacted. In an attempt to obtain further information about the quantitative nature of dye formation, some additional experiments were carried out. At the end of each reaction, and before extraction, 10 ml of a saturated solution of ascorbic acid were added to the reaction product. This served the purpose of destroying any excess of reagent and also reducing some oxidised forms of tocopherol. The results (see Table I) confirm that the coupling reaction itself is incomplete in potassium hydroxide, even at the lowest levels of γ-tocopherol used. It is apparent that a competitive oxidation takes place during reaction, the diazo compound being the oxidant. The extent of the oxidation reaction, as might be expected, increases as the proportion of γ-tocopherol increases, and the product of oxidation can—at least partly—be reduced by ascorbic acid. It has been shown by Harrison, Gander, Blakley and Boyer[12] that the only identifiable oxidation product of α-tocopherol reducible to α-tocopherol by ascorbic acid in the absence of mineral acid is the *soi-disant* "tocoperoxide" (recently shown by Martius and Eilingsfeld[13] to be a hemi-ketal, not an oxide); α-tocopherylquinone is only converted to α-tocopherolhydroquinone by ascorbic acid in the absence of mineral acid. Since the first stages of γ-tocopherol oxidation parallel those in the oxidation of α-tocopherol,[14] the results lead to the conclusion that, during the coupling reaction of γ-tocopherol in ethanolic potassium hydroxide, part of the γ-tocopherol is converted to a γ-tocopherethylacetal. It is further possible that some of the γ-tocopherol is converted to an irreversible oxidation product that cannot be measured. As Weisler *et al.* have found that the sodium carbonate reaction yields in fact slightly less dye than does the potassium hydroxide reaction, it can be concluded that the former reaction does indeed oxidise at least similar amounts of the tocopherol to such an irreversible oxidation product—perhaps the quinone—since considerably smaller amounts of γ-tocopherol can be recovered after reduction with ascorbic acid (see Table I).

η-TOCOPHEROL—

Table I shows the results found with η-tocopherol. At the 100-μg level, reaction is complete under both conditions of pH. The extinction of the dye is identical with that from γ-tocopherol, and the reactions of the two tocopherols are obviously closely similar at low tocopherol levels. At higher levels appreciable amounts of η-tocopherol could be recovered from both reactions. Both coupling and oxidation reactions are slower than those of γ-tocopherol.

δ-TOCOPHEROL—

Chromatography of the reaction product—Weisler *et al.* found that the coupling of δ-tocopherol with diazotised dianisidine was markedly influenced by pH, a fact they were unable to explain, since the reaction of γ-tocopherol was barely affected. We repeated the two reactions on 100-μg amounts of δ-tocopherol, and again could not reproduce the "$E_{1cm}^{1\%}$ values" found by these workers. The values found from the potassium hydroxide and sodium carbonate reactions were 131 and 215, respectively, compared with 129 and 301 found by Weisler *et al.* The reactions were studied in more detail by chromatographing the products on paper impregnated with liquid paraffin, with 75 per cent. ethanol as mobile phase. Only the tocopherols migrate in this system, the dyes and other reaction products staying on the starting line. The results are summarised in Table I. It was observed that even with only 100 μg of tocopherol, reaction did not go to completion in potassium hydroxide solution, unchanged δ-tocopherol being recovered (unlike the analogous reaction on γ-tocopherol). In sodium carbonate solution, 100 μg reacted completely, but larger amounts did not. Further, when ascorbic acid was added to the potassium hydroxide reaction mixture at the end of the reaction time, even larger amounts of tocopherol could be recovered. It seems clear that the results of Weisler *et al.* can be partly explained by the lack of reactivity of δ-tocopherol to coupling in strong alkali. This inhibition of coupling is discussed further under "Reaction of Tocopherols with 2 : 6-Dichloro-*p*-benzoquinone-4-chloroimine," p. 301, but it may be noted here that the diminished reactivity of δ-tocopherol has been found in other reactions.[5,15]

However, the greatly increased extinction of the dye from the carbonate reaction does not depend solely on more efficient coupling. Chromatographic analysis of the total product from the coupling of δ-tocopherol in sodium carbonate solution, by adsorption chromatography on paper treated with zinc carbonate, revealed four coloured bands. The R_F values were 0, 0·05, 0·40 and 0·67. These dyes were so strongly adsorbed that they could not be extracted with the most polar solvents and could only be examined after destruction of the zinc carbonate with acid. The acid treatment destroyed the two slower bands, and they could not be studied further. However, they were of low intensity and did not form a major part of the reaction product.

Determination of the two dyes—Some 100-μg portions of δ-tocopherol were treated with diazotised dianisidine under each condition of pH. The total product from each reaction was chromatographed on zinc carbonate paper. The first band (R_F 0·67) was rose-pink in colour and the second band (R_F 0·40) was blue. The two bands were cut out separately, and each was eluted with 4 ml of a mixture of methanol and concentrated hydrochloric acid (10 + 1) in a stoppered test-tube. After addition of 3 ml of light petroleum (boiling range 80° to 100° C) to each tube, the dyes were extracted and their absorptions measured. The results are shown in Table II and indicate that, in sodium carbonate solution, a second coloured product (of which only a small amount is in the product from potassium hydroxide solution) is formed in considerable quantity. The two dyes have different absorption maxima, thus accounting for the wavelength shift recorded by Weisler *et al.* After appropriate calculations, the figures in Table II give "$E_{1cm}^{1\%}$ values" (based on original weight of tocopherol) of 92 and 147 for the two reactions. Since, as has been stated, there are two other coloured products included in the total extinction measurements, these figures are in fair agreement with our own gross extinction values. These facts would appear to explain the results of Weisler *et al.* on δ-tocopherol.

Nature of the two dyes from δ-tocopherol—The faster band from δ-tocopherol corresponds chromatographically to the single dye from γ- or η-tocopherol. The slower second band could arise by one of two mechanisms. Either δ-tocopherol reacts with tetra-azotised dianisidine at the lower pH to give a bis-azo compound in addition to the normal product or the second dye

is formed by entry of a second diazonium moiety into position 7 of the tocopherol molecule. Experiments were carried out to study these two possibilities. A solution of authentic tetra-azotised o-dianisidine was prepared and allowed to react with δ-tocopherol at each condition of pH. Qualitatively and quantitatively the results were identical with those from normal coupling, implying that bis-azo dye formation played no part in formation of the slow band. Since it was possible that reaction rate influenced preferential coupling with either diazotised or tetra-azotised molecules, the reaction products from η-tocopherol were then analysed by chromatography on paper treated with zinc carbonate. Only one band was observed, in spite of the fact that this tocopherol couples as sluggishly as does δ-tocopherol.

TABLE II

ABSORPTION OF THE TWO DYES FROM 100 μg OF δ-TOCOPHEROL

			$\lambda_{max.}$	Extinction at 510 mμ after—	
				KOH coupling	Na$_2$CO$_3$ coupling
Slower band	..	..	520	0·045	0·214
Faster band	..	..	495	0·230	0·226

To test the alternative hypothesis, some experiments were carried out in which δ-tocopherol was coupled with diazotised p-nitroaniline. Under all conditions of pH tried, only one dye was observed on adsorption paper chromatograms. However, in view of the results with the tetra-azotised dianisidine reagent, it is still possible that the second dye from δ-tocopherol is produced as a result of abnormal coupling in the 7-position by a diazotised dianisidine molecule, in which event it is possible that diazotised p-nitroaniline only yields one dye because it is so reactive as to couple equally rapidly in the 5-position of δ- or γ-tocopherol.

β- AND ε-TOCOPHEROL—

Even the most reactive diazonium salts do not couple with β-tocopherol in solution to an observable extent. However, when a spot containing 5 to 20 μg of β-tocopherol is put on a chromatographic paper impregnated with zinc carbonate and sprayed first with dilute sodium carbonate solution and then with the diazotised dianisidine reagent, a characteristic orange-brown colour is produced. This reaction is also given by ε-tocopherol, but not by α-tocopherol. The colour is, therefore, not apparently produced by oxidation. It may indeed be due to a coupling reaction occurring at a rate many times slower than that with γ- or δ-tocopherol. (For a discussion on orientation in aromatic systems and the effect of differential rates in diazo coupling, as affected by resonance-energy differences, see Wheland.[16])

In spite of the absence of appreciable coupling in solution, it was found that β-tocopherol could not be recovered quantitatively after the dianisidine reaction. Large and variable amounts of this tocopherol were always destroyed, presumably by oxidation; the results are shown in Table I. As in the example of γ-tocopherol, oxidation of small amounts in sodium carbonate solution is rapid, complete and irreversible, but oxidation is incomplete in potassium hydroxide solution, and much of the oxidation product must be the intermediate tocophero-ketal. When larger amounts of tocopherol were used in potassium hydroxide solution, recoveries were appreciable but not quantitative.

Results with both ε- and α-tocopherol were similar, but are not shown in Table I.

REACTION OF TOCOPHEROLS WITH 2:6-DICHLORO-p-BENZOQUINONE-4-CHLOROIMINE (GIBBS' REAGENT)

The unsatisfactory nature of the dianisidine reaction led us to the study of other reagents that, it was hoped, might remove γ- and η-tocopherol quantitatively under conditions from which β- and ε-tocopherol could be recovered (after reduction, if necessary). Mohlau[17] first prepared quinonechloroimines and found that they formed deep-blue indophenols with a number of phenols. Gibbs[18] studied the reaction extensively and introduced the fairly stable 2:6-dichloro-p-benzoquinone-4-chloroimine as a general reagent for phenols. Gibbs believed that only phenols with unsubstituted *para* positions would react. However, we have found that this chloroimine reacts positively and immediately to give indophenols with certain phenols that have exceptionally active *ortho* positions, including 2-tetralol, 2-naphthol, half-ethers of hydroquinones and γ-, δ- and η-tocopherol. β-Tocopherol and ε-tocopherol

do not so react.   The differential reactions of β-, γ-, ϵ- and η-tocopherol were then investigated fully.

GENERAL CONDITIONS OF REACTION—

A solution of 100 to 1000 μg of tocopherol in 10 ml of ethanol was brought to the requisite pH by adding 6 ml of alkali or 6 ml of an appropriate borate buffer solution.   A 3-ml portion of a freshly prepared 0·1 per cent. w/v solution of the chloroimine in ethanol was added, and the suspension was shaken well and set aside for 3 minutes.   At the end of this time, 10 ml of an 8·5 per cent. w/v solution of ascorbic acid were added.   After dilution with more water, the solution was extracted with light petroleum (boiling range 40° to 60° C).   The washed organic layer was evaporated at low temperature and the residue was dissolved in 1 to 2 ml of benzene.   Chromatographic analysis of the reaction products was carried out as described on p. 300.

γ-TOCOPHEROL—

γ-Tocopherol gives immediately a deep blue colour with the reagent.   The dye is readily extractable, without prior acidification, into light petroleum to give a red solution and has $\lambda_{max.}$ at 528·5 mμ.   The product is presumably an o-phenolindophenol of formula—

(I)   (II)
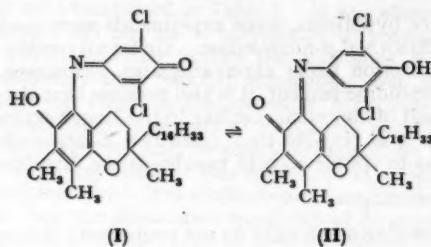
The indophenol is easily separable from the tocopherol by adsorption chromatography on paper treated with zinc carbonate.   It migrates as a greenish blue band to a position between α- and γ-tocopherol.

QUANTITATIVE STUDIES—

Table III shows the results obtained for the reaction of γ-tocopherol.   The chief fact that emerges is that the main factor influencing reaction is pH; indophenol formation is maximal at pH 9, falling off sharply as the strength of alkali is increased.   This effect of pH is in accordance with the findings of Lothrop,[19] who studied the coupling of substituted phenols with diazotised nitroaniline.   The second factor that influences indophenol formation is (as in the dianisidine reaction) the amount of tocopherol taken.   Even at pH 9, no more than about 250 μg of γ-tocopherol can be completely removed by the procedure, and even in these circumstances small amounts of tocopherol are often recovered.   When the amount of tocopherol is increased to 1000 μg, 5 to 10 per cent. is recoverable after the reaction at pH 9.

Several other factors were studied in attempts to render the reaction of γ-tocopherol more complete.   These included time of reaction, volume of solution, amount of chloroimine and choice of reducing agent to stop the reaction.   No discernible effect was produced by any of these factors and particulars of these experiments have therefore not been included in Table III.

δ- AND η-TOCOPHEROL—

The reactions of these two tocopherols with chloroimine at pH 9 were investigated, and the results are summarised in Table III. δ-Tocopherol reacts more slowly than γ-tocopherol and greater amounts of the former are recovered at the end of the 3-minute reaction time (the reagent is completely destroyed by the alkali within this time).   The indophenol from δ-tocopherol runs to the same position on zinc carbonate paper as that from γ-tocopherol. Since R values of phenols in adsorption chromatography are affected by $ortho$substitution,[20] this would indicate that structure (II) above is the preferred form of these indophenols. η-Tocopherol reacts even more slowly than δ-tocopherol.   Recoveries are not apparently affected by the amount of tocopherol used (unlike the dianisidine reaction).

TABLE III

RECOVERY OF TOCOPHEROLS AFTER REACTION WITH CHLOROIMINE

	Amount of tocopherol taken, µg	Alkali used	No. of tests	Recovery, %
γ-Tocopherol	254		6	0 to 2
	508	Na₂CO₃ (5 per cent.)	4	0 to 2
	1065		3	9 to 15
	1065	KOH (5 per cent.)	1	15
	1065	KOH (50 per cent.)	1	68
	246		4	0 to 2
	615	Borate (pH 9·0)	1	9
	1260		4	5 to 6
	1260	Borate (pH 8·0)	1	22
	1260	Borate (pH 8·5)	1	15
	1260	Borate (pH 9·2)	1	8·5
	1260	Borate (pH 10·0)	1	84
δ-Tocopherol	160		1	21
	800	Borate (pH 9·0)	1	14
	1600		1	15
η-Tocopherol	130	Borate (pH 9·0)	1	53
	1300		1	31
β-Tocopherol	97	Na₂CO₃ (5 per cent.)	4	50 to 93
	97	Borate (pH 9·0)	6	46 to 64
	1357	Na₂CO₃ (5 per cent.)	3	39 to 86
	1357	Borate (pH 9·0)	3	51 to 91

β-TOCOPHEROL—

Various amounts of β-tocopherol were treated with chloroimine under different conditions and the recoveries studied by chromatographic analysis. The results are summarised in Table III. The recoveries of β-tocopherol were not reproducible, did not depend significantly on the amount taken for reaction and, in general, were much higher than those from the reactions with diazotised dianisidine. In attempts to improve the reproducibility and to make the recovery of β-tocopherol more quantitative, the use of other reducing agents at the end of the reaction was investigated. These agents included pyrogallol, 2-naphthol and sulphurous acid. Reductive cyclisation procedures (ascorbic acid - hydrochloric acid and stannous chloride - hydrochloric acid) were also tried. None of the methods proved satisfactory, and the results of these experiments have not been recorded in Table III.

CONCLUSIONS

Neither the reaction with diazotised dianisidine nor the reaction with Gibbs' reagent (both of which depend on differential coupling at the reactive 5-position) can effectively separate β- and ε-tocopherol from their mixtures with γ- and η-tocopherol, respectively.

REFERENCES

1. Brown, F., *Biochem. J.*, 1952, **51**, 237; 1952, **52**, 523.
2. Eggitt, P. W. R., and Ward, L. D., *J. Sci. Food Agric.*, 1953, **4**, 176 and 569.
3. Green, J., Marcinkiewicz, S., and Watt, P. R., *Ibid.*, 1955, **6**, 274.
4. Marcinkiewicz, S., and Green, J., *Analyst*, 1959, **84**, 304.
5. Quaife, M. L., *J. Biol. Chem.*, 1948, **175**, 605.
6. Baxter, J. G., Robeson, C. D., Taylor, J. D., and Lehman, R. W., *J. Amer. Chem. Soc.*, 1943, **65**, 918.
7. Weisler, L., Robeson, C. D., and Baxter, J. G., *Anal. Chem.*, 1947, **19**, 906.
8. Quaife, M. L., *J. Amer. Chem. Soc.*, 1944, **66**, 308.
9. Harris, P. L., Quaife, M. L., and Swanson, W. J., *J. Nutrit.*, 1950, **40**, 367.
10. Lehman, R. W., *Methods of Biochemical Analysis*, 1955, **2**, 153.
11. Green, J., *J. Sci. Food Agric.*, 1958, **9**, 801.
12. Harrison, W. H., Gander, J. E., Blakley, E. R., and Boyer, P. D., *Biochim. Biophys. Acta*, 1956, **21**, 150.
13. Martius, C., and Eilingsfeld, H., *Annalen*, 1957, **607**, 159.
14. Eggitt, P. W. R., and Norris, F. W., *J. Sci. Food Agric.*, 1955, **6**, 689.
15. British Patents 639,011 and 676,853.
16. Wheland, G. W., "Resonance in Organic Chemistry," J. Wiley & Sons Inc., New York, 1955.
17. Mohlau, R., *Ber.*, 1883, **16**, 2843.
18. Gibbs, H. D., *J. Biol. Chem.*, 1927, **72**, 649.
19. Lothrop, W. C., *J. Amer. Chem. Soc.*, 1940, **62**, 132.
20. Green, J., and Marcinkiewicz, S., *Nature*, 1955, **176**, 1172.

NOTE—Reference 4 is to Part II of this series.

The Complete Analysis of Tocopherol Mixtures

Part II. The Separation of Nitroso-tocopherols by Paper Chromatography and their Determination

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A method is described for the complete separation and quantitative determination of all the known tocopherols. The formation of nitroso-derivatives by β-, γ-, δ-, ϵ-, and η-tocopherol and their two-dimensional paper chromatography have been studied. The six nitroso-derivatives (δ-tocopherol forms two) can be separated on one chromatogram. The nitroso-derivatives can then be determined by a modification of the ferric chloride - dipyridyl oxidation reaction, in which the extinction at 520 mμ is measured after 60 minutes. The sensitivity of the method is high; 1 μg of β-tocopherol can be detected in 100 μg of γ-tocopherol. The method has been applied to synthetic mixtures of the tocopherols and to natural oils. β-Tocopherol has been found in the γ-tocopherol fractions of maize oil and cottonseed oil.

As shown in Part I of this series,[1] differential coupling reactions do not appear to be suitable for the separation of β- and ϵ-tocopherol from γ- and η-tocopherol, respectively. Quaife[2] found that the nitroso-derivatives of β-, γ- and δ-tocopherol could be separated by adsorption on zinc carbonate columns and determined by measuring their extinctions at 410 mμ. This wavelength is at or near the absorption maximum of the nitroso-derivatives studied by Quaife. We have now re-investigated the nitrosation of tocopherols (including ϵ- and η-tocopherol, which were unknown to Quaife) and have studied the separation of the nitroso-derivatives by paper-chromatographic methods.

EXPERIMENTAL

MATERIALS AND GENERAL METHODS—

The γ-, δ- and ϵ-tocopherol used were pure distilled samples prepared from natural sources; the β- and η-tocopherol were pure synthetic specimens.

Chromatographic methods and the determination by the modified Emmerie - Engel procedure were as described in Part I of this series and previously.[3],[4],[5]

CHROMATOGRAPHIC SEPARATION OF NITROSO-TOCOPHEROLS—

In a preliminary series of experiments, 100 to 500-μg amounts of each tocopherol were nitrosated by Quaife's method. After extraction of the product into light petroleum, a suitable amount (20 to 50 μg) of each nitroso-derivative was chromatographed on zinc carbonate paper by the ascending-solvent method, with a 30 per cent. v/v solution of benzene in *cyclo*hexane as developing solvent. After 1 hour, R values were measured to the front of each band, as is usual in adsorption chromatography. Similar amounts of the nitroso-tocopherols were then chromatographed on zinc carbonate paper impregnated with liquid paraffin, also by the ascending-solvent method, and with 93 per cent. v/v ethanol in water as mobile phase. In 3 hours, good separation was obtained, and R_F values were measured to the middle of each band, as is conventional. The R and R_F values are shown in Table I.

TABLE I

R AND R_F VALUES OF THE NITROSO-TOCOPHEROLS

Nitroso-derivative				R value	R_F value
β-Tocopherol	..	..	..	0·70	0·415
γ-Tocopherol	..	..	..	0·77	0·192
ϵ-Tocopherol	..	..	..	0·70	0·600
η-Tocopherol	..	..	..	0·70	0·313
δ-Tocopherol (7-nitroso)	..	..	0·25	0·791
δ-Tocopherol (5-nitroso)	..	..	0·46	0·480

As indicated by the tabulated values, the nitroso-derivatives of β-, γ- and δ-tocopherol separate by adsorption on zinc carbonate paper as they do on adsorption columns. The

nitroso-derivatives of ϵ- and η-tocopherol move with the nitroso-derivatives of β- and γ-tocopherol and are inseparable from them, all bands being rather diffuse. δ-Tocopherol formed two easily separable nitroso-derivatives; we have assigned them the structures of 7-nitroso-δ-tocopherol and 5-nitroso-δ-tocopherol. It is hoped that an account of the isolation and infra-red analysis of the two compounds will be published elsewhere. However, the evidence provided by their R and R_F values, $E_{1\,cm}^{1\%}$ values, adsorption maxima and the proportions of the two substances formed in the nitrosation reaction confirm the assigned structures. Quaife,[2] in a parenthetical note, remarks that δ-tocopherol migrated as one or two rings on zinc carbonate - Celite columns.

On reversed-phase partition chromatography, the nitroso-derivatives migrated as sharply defined yellow bands. A mixture of all six nitroso-tocopherols can be separated by complete two-dimensional chromatography, and Fig. 1 shows diagrammatically a typical result. If ordinary Whatman No. 1 filter-paper previously impregnated with zinc carbonate is used to support the liquid paraffin phase, little effect on R_F values is observed, but the bands are orange in colour. If the chromatogram is immersed in concentrated sodium hydroxide solution, the nitroso-derivatives turn pink. The 7-nitroso-derivatives resume their original yellow colour when the paper is washed and dried, but the 5-nitroso-derivatives stay pink. This provides a delicate test for the latter derivatives and, incidentally, provides further evidence for the structure of the two bands from δ-tocopherol; only the slower band gives a permanent pink colour with alkali.

In the studies described below, reversed-phase paper chromatography only was used as a method of analysis.
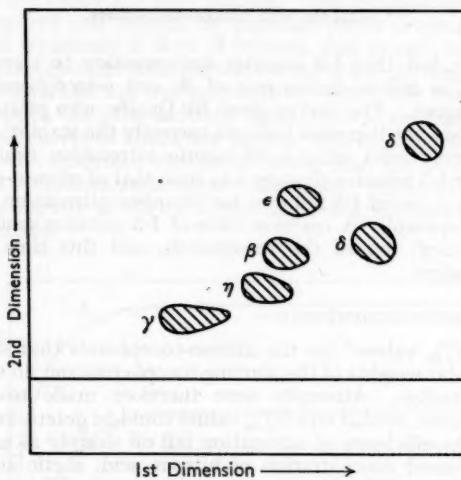

Fig. 1. Two-dimensional paper chromatogram
of six nitroso-derivatives

AMOUNT OF TOCOPHEROL NITROSATED—

Nitrosation was complete when amounts up to 600 μg were used. Some γ- and δ-tocopherol could be recovered unchanged when the reaction products from 1-mg portions were chromatographed. In view of this, 600 μg was the maximum amount of tocopherol taken for nitrosation in all further experiments.

TIME OF NITROSATION—

The reaction time used by Quaife was 1 minute, but she suggested that 2 minutes might be preferable when dealing with δ-tocopherol alone. We have studied the effect of different nitrosation times on the formation of nitroso-derivatives of β-, γ- and δ-tocopherol. The tocopherol (about 500 μg) was nitrosated, and the reaction was stopped by the addition of alkali. The nitroso-derivative was extracted into light petroleum, and a suitable aliquot

from each extract was taken for chromatographic analysis. After 1 hour, the nitroso-deriv-ative had separated sufficiently; the yellow band was cut out and eluted by gentle swirling in a test-tube with 10 ml of light petroleum. The extinction was then measured at the absorp-tion maximum (see below). The results (see Table II) show that nitrosation of γ-tocopherol

TABLE II

EFFECT OF NITROSATION TIME ON NITROSO-TOCOPHEROLS

	Amount of tocopherol nitrosated, μg	Nitrosation time, minutes	Net extinction at λ_{max}
β-Tocopherol ..	560	1·0	0·301
		1·5	0·360
		2·0	0·358
		5·0	0·236
		60·0	0
γ-Tocopherol ..	540	1·0	0·308
		1·5	0·276
		5·0	0·273
		60·0	0·008
δ-Tocopherol ..	567	1·0	0·525*
		1·5	0·550
		2·0	0·549

* Total for both nitroso-derivatives.

is complete in 1 minute, but that 1·5 minutes are necessary to complete the reaction for β- and δ-tocopherol. The nitroso-derivatives of β- and γ-tocopherol are unstable in the presence of excess of reagent. The curves given by Quaife, who plotted total yellow colour against time of reaction, do not therefore indicate correctly the stability of the nitroso-deriva-tives, which in fact hardly exist after a 60-minute nitrosation time. The extinction of nitroso-γ-tocopherol over 1·5 minutes changes less than that of nitroso-β-tocopherol. ϵ-Toco-pherol, like β-tocopherol, required 1·5 minutes for complete nitrosation, whereas η-tocopherol behaved more like γ-tocopherol. A reaction time of 1·5 minutes could therefore be used, with little loss of accuracy, for all the tocopherols, and this time was adopted in the experiments described below.

ABSORPTION OF THE NITROSO-TOCOPHEROLS—

Quaife has given "$E_{1\,cm}^{1\%}$ values" for the nitroso-tocopherols that she studied, but these were based on the molecular weights of the starting tocopherols and do not in fact refer to the nitroso-tocopherols themselves. Attempts were therefore made to prepare the nitroso-tocopherols in larger amounts, so that true $E_{1\,cm}^{1\%}$ values could be determined on pure specimens. However, not only did the efficiency of nitrosation fall off sharply as amounts of tocopherol were increased, but increased concentration of nitrous acid, acetic acid or the tocopherol itself resulted in degradation of the nitroso-derivative formed. Even in experiments on as little as 10 mg of tocopherol, and with a five-fold increase in concentration of reagents, only impure material could be isolated by chromatography on zinc carbonate columns. Never-theless, it was possible to isolate milligram amounts of almost pure nitroso-tocopherols by combining the products of several analytical nitrosations and then separating the nitroso-derivatives on partition paper chromatograms. The nitroso-derivatives were dissolved in light petroleum and their extinctions measured. The "$E_{1\,cm}^{1\%}$ values" for the nitroso-reaction are shown in Table III, these values referring to the weights of starting tocopherol. The experimental work (described later) on the quantitative aspects of the reactions makes it likely that the figures recorded are indeed close to the true $E_{1\,cm}^{1\%}$ values of the nitroso-tocopherols.

The values found were in excellent agreement with those of Quaife when a nitrosation time of 1 minute was used. After 1·5-minute nitrosations, there were slight differences. The nitroso-derivatives of ϵ- and η-tocopherol have absorption maxima almost identical with those of the nitroso-derivatives of β- and γ-tocopherol, respectively. The "$E_{1\,cm}^{1\%}$ values" for the two nitroso-derivatives from δ-tocopherol cannot be obtained without a knowledge of the proportion of the two substances. If, however, it is assumed that they have approximately

the same extinction, then the proportion of the 5-nitroso-derivative to the 7-nitroso-derivative varied between 2·5 and 4 to 1, in a series of nitrosations.   Synthetic and natural specimens of δ-tocopherol behaved identically.

TABLE III

$E_{1 cm}^{1\%}$ VALUES FOR THE NITROSO-TOCOPHEROLS
The nitrosation time was 1·5 minutes

Nitroso-derivative				$\lambda_{max.}$, mμ	"$E_{1 cm}^{1\%}$"
β-Tocopherol	..	..	..	410	43·5*
γ-Tocopherol	..	..	..	415	51·3†
ε-Tocopherol	..	..	..	410	43·5
η-Tocopherol	..	..	..	415	48·8

* Value after 1-minute nitrosation is 37·6.
† Value after 1-minute nitrosation is 54·7.

THE REACTION OF NITROSO-TOCOPHEROLS WITH FERRIC CHLORIDE—

The determination of small amounts of the nitroso-tocopherols by measuring their absorptions at 410 mμ is difficult because of their low extinctions.   It was found that the nitroso-tocopherols reduced ferric chloride and gave the typical red colour with the Emmerie - Engel reagent.   Before investigating the reaction as a method of quantitative determination, the characteristics of the oxidation were studied.   The nitroso-derivatives of β-, γ-, ε- and η-tocopherol were prepared and isolated by partition paper chromatography.   About 50 μg of each were dissolved separately in 3 ml of ethanol, and to each solution was added 0·5 ml of 0·2 per cent. w/v ferric chloride solution and 0·5 ml of 0·5 per cent. w/v dipyridyl solution, both in ethanol.   A blank solution was prepared by adding the reagents to 3 ml of ethanol.   The solutions were kept in the dark and their extinctions were measured at 520 mμ at intervals.   Fig. 2 shows the curves of extinction against time for the different nitroso-derivatives.   It was observed that the oxidation was much slower than the oxidation of the tocopherols (complete in less than 2 minutes under these conditions).   After 60 minutes, the final colour was stable, increasing slightly as the corresponding blank increased; the net extinction was constant and reproducible.
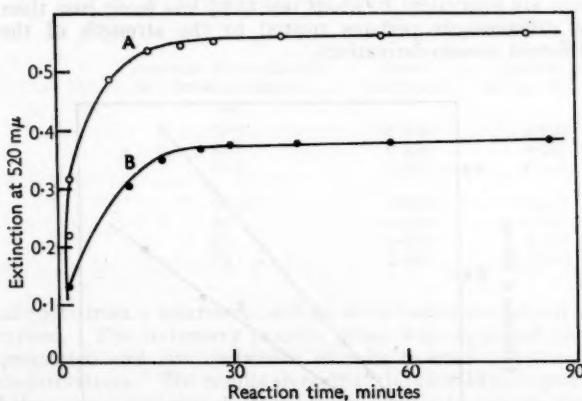
Fig. 2.   Oxidation curves for β-, γ-, ε- and η-tocopherol nitroso-derivatives: curve A, β- and ε-tocopherol; curve V, γ- and η-tocopherol

QUANTITATIVE STUDIES—

A fully quantitative study of the proposed method involves a study of (a) the nitrosation, (b) the chromatographic separation, and (c) the determination.

These three steps are inter-related, in that no single one can be adequately studied without performing the other two; it was therefore necessary to carry out the quantitative studies with due regard to the logical requirements of the problem and avoiding argument in a circle. The method adopted was to prepare solutions of the purified nitroso-tocopherols by pooling the results of several analytical nitrosations and to study the determination step and the paper-chromatographic step separately on these solutions. The exact purity of the solutions was not ascertainable by external criteria not implicit in the analytical procedure, but the results obtained did not depend on knowledge either of their purity or of the reproducibility of the nitrosation reaction. The reproducibility of the nitrosation itself could then be studied independently.

Determination of the nitroso-tocopherols—Three separate 500-μg portions of β-tocopherol were nitrosated. The nitroso-derivative was extracted from each reaction product with 15 ml of light petroleum (boiling range 40° to 60° C). The combined extracts were washed three times with water and then made up to 50 ml in a calibrated flask. The extinction was measured at 410 mμ; "$E_{1\,cm}^{1\%}$" was 43·5. The same procedure was carried out with 1·5 mg of γ-, ϵ- and η-tocopherol. "$E_{1\,cm}^{1\%}$" values for the three nitroso-derivatives at 410 mμ were found, in these experiments, to be 54·7, 43·8 and 54·7, respectively.

Each solution was evaporated to dryness under reduced pressure (without allowing the temperature to rise above 30° C), and each nitroso-tocopherol was re-dissolved in 6 ml of ethanol. Because of the known sensitivity of the nitroso-tocopherols to heat, the quantitative nature of the step was checked by taking part of each solution, diluting again with light petroleum and re-determining the $E_{1\,cm}^{1\%}$ value. No change had occurred in any of the four solutions. From each ethanolic solution, aliquots were taken by micro-capillary pipette, and each aliquot was dissolved in 3 ml of ethanol in a small stoppered test-tube. Each solution was then treated by the usual modified Emmerie - Engel procedure and then measured against the blank solution after a 60-minute reaction time. Readings at 520 mμ were plotted against micrograms of original tocopherol taken for nitrosation; as shown in Fig. 3, the relationships were linear. The spectrophotometric factors for the over-all nitroso-reaction and ferric chloride oxidation are 84 $\pm$ 4 for β- and ϵ-tocopherol and 128 $\pm$ 5 for γ- and η-tocopherol. These may be compared with spectrophotometric factors determined for the direct oxidation of the parent tocopherols, which are 96 and 90, respectively. Nitroso-β-tocopherol and nitroso-ϵ-tocopherol are therefore equivalent to rather more ferric iron than are their parent tocopherols, probably owing to some slow oxidation of the nitroso-group, whereas nitroso-γ-tocopherol and nitroso-η-tocopherol are equivalent to about one-third less ferric iron than are their parent tocopherols. The difference is perhaps related to the strength of the intra-molecular chelation in the different nitroso-derivatives.
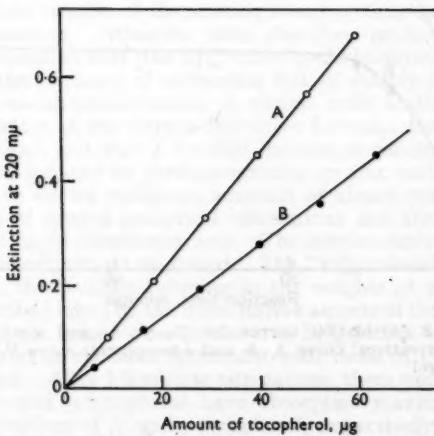

Fig. 3. Calibration graphs for β-, γ-, ϵ- and η-tocopherol:
curve A, β- and ϵ-tocopherol; curve B, γ- and η-tocopherol

Paper chromatography—With the aid of the ferric chloride determination procedure it was possible to investigate the quantitative nature of the paper-chromatograhic step on small amounts of nitroso-tocopherols.   The four nitroso-derivatives were isolated in 1 to 2 mg amounts, as described above.   The reproducibility of "$E_{1\,cm}^{1\%}$." values on the solutions before and after isolation showed that no appreciable loss occurred during the chromatographing of these large amounts.   Each nitroso-derivative was dissolved at a series of concentrations in portions of light petroleum between 1 and 10 ml.   From each solution, aliquots were taken containing between 10 and 100 μg of material, dissolved in from 10 μl to 6 ml of solution. These aliquots were chromatographed for different lengths of time on paper impregnated with liquid paraffin.   After the chromatographic run, the nitroso-derivative was cut out from each paper, eluted in a test-tube with 3 ml of ethanol and determined by the modified ferric chloride procedure.   The results are shown in Tables IV and V.   Table IV shows the

TABLE IV

PARTITION CHROMATOGRAPHY OF NITROSO-β-TOCOPHEROL

Extinction at 520 mμ

| Volume of solution chromatographed, μl | Plain paper— | | Zinc carbonate paper after 2-hour run | Direct oxidation |
	after 30-minute run	after 2-hour run		
10	0·128	0·124	0·127	0·131
20	0·248	0·247	0·246	0·258
30	0·369	0·374	0·360	0·377
40	0·510	0·512	0·498	0·512
50	0·648	0·642	0·636	0·644
60	0·901	0·900	0·902	0·898
70	1·002	1·000	1·000	1·009

TABLE V

RECOVERY OF NITROSO-TOCOPHEROLS FROM PAPER CHROMATOGRAMS AFTER SPOTTING AT DIFFERENT DILUTIONS

Extinction at 520 mμ

	Amount of tocopherol, as nitroso derivative, μg	Direct oxidation	Spotted at 220 μg per ml	Spotted at 15 μg per ml
β-Tocopherol	17·6	0·200	0·190	0·165
	35·2	0·402	0·387	0·279
	52·8	0·602	0·577	0·416
γ-Tocopherol	10	0·082	0·077	0·078
	25	0·198	0·197	0·175
	50	0·371	0·366	0·358
	75	0·552	0·535	0·515

recoveries when aliquots from a solution of 500 μg of nitroso-β-tocopherol in 1 ml of benzene were chromatographed.   The stationary paraffin phase was supported both on plain paper and on paper impregnated with zinc carbonate, in order to study the effect of the basic zinc salt on the nitroso-derivatives.   The results show that the chromatographic run itself did not cause any loss of the nitroso-tocopherol, since almost identical amounts were recovered after 30 minutes and 2 hours on the paper.   However, there was normally a small but reproducible difference between determinations of identical amounts before and after chromatography, which was eventually traced to losses during the spotting procedure.   In a series of experiments designed to show this more clearly, various amounts of the above-mentioned nitroso-β-tocopherol solution were spotted on paper by the normal spotting technique and immediately eluted with ethanol and determined.   The average factor found was 90 $\pm$ 4, which shows a 6 per cent. loss on spotting.   In another series of experiments, less than 2 per cent. loss occurred when nitroso-γ-tocopherol was spotted in amounts from 10 to 100 μg.   Further

experiments were then carried out to study the factors affecting the loss; the results are shown in Table V. Two factors were found to influence the loss on spotting.

(i) The concentration of the spotting solution. In general, the more dilute the spotting solution the greater the loss—as much as 30 per cent. of nitroso-β-tocopherol may be destroyed if solutions containing less than 10 μg per ml are used for spotting. However, if the concentration of nitroso-tocopherol is at least 250 μg per ml, the loss is no more than a few per cent. The type of solvent did not appear to influence the recovery.

(ii) The nitroso-derivative itself. It was constantly observed that nitroso-γ-tocopherol was more stable than nitroso-β-tocopherol. In general, wide variations in spotting technique and amount used did not affect the recovery of nitroso-γ-tocopherol by more than 10 per cent. Exactly similar findings were made when the nitroso-derivatives of ϵ- and η-tocopherol were compared; the latter was more stable than the former.

As a result of these experiments it was concluded that the nitroso-derivatives should, for quantitative separation and determination, be spotted from solutions containing at least 250 μg per ml. Under these conditions, the best practical spectrophotometric factors for nitroso-β-tocopherol and for nitroso-ϵ-tocopherol appear to be about 92 and for nitroso-γ-tocopherol and nitroso-η-tocopherol about 130.

Nitrosation—To test the reproducibility of the nitrosation, amounts of β- and γ-tocopherol between 100 and 500 μg were used. The nitroso-derivatives were chromatographed and determined by the ferric chloride procedure. The variation was not found to be greater than that already inherent in the chromatographic procedure. By means of the factors of 92 and 130, respectively, recoveries of over 95 per cent. were consistently achieved, provided that the final solutions used for spotting contained at least 250 μg of nitroso-tocopherol per ml.

METHOD

REAGENTS—

Acetic acid—Analytical-reagent grade.

Sodium nitrite solution, 2 per cent.

Potassium hydroxide solution—Dissolve 20 g of potassium hydroxide in 100 ml of water.

Light petroleum (boiling range 40° to 60° C)—Analytical-reagent grade.

Light petroleum (boiling range 60° to 80° C)—Analytical-reagent grade.

Ethanol, absolute—Purify by distilling 1 litre of ethanol from a mixture of 1 g of potassium permanganate and 2 g of potassium hydroxide.

Liquid paraffin solution—A 3 per cent. v/v solution of liquid paraffin, B.P., in light petroleum.

Ethanol for chromatography—A 93 per cent. v/v aqueous solution.

Ferric chloride reagent solution—A 0·2 per cent. w/v solution of analytical-reagent grade ferric chloride, $FeCl_3.6H_2O$, in ethanol. The solution must be prepared directly in the amber-coloured bottle in which it is to be stored. It should not be exposed even to moderate daylight at any time and should be stored in complete darkness. Failure to carry out these precautions will lead to unsatisfactory results.

2:2'-Dipyridyl solution—A 0·5 per cent. w/v solution of analytical-reagent grade 2:2'-dipyridyl in ethanol.

PROCEDURE—

Nitrosation—Carry out this reaction in a darkened room under artificial light. Dissolve not more than 600 μg of the mixture of β- and γ-tocopherol or of ϵ- and η-tocopherol in 5 ml of ethanol in a small separator. Add 0·2 ml of acetic acid, and mix by swirling. Add, from a fast-delivery pipette, 3 ml of 2 per cent. sodium nitrite solution, and again mix by swirling vigorously. Start a stop-watch simultaneously with the addition of the nitrite, and, after exactly 90 seconds, add 2 ml of potassium hydroxide solution, and shake to mix. Add 10 to 15 ml of water and 20 ml of light petroleum (boiling range 40° to 60° C). Extract the nitroso-tocopherol into the light petroleum by shaking vigorously for at least 1 minute, after which no pink colour should remain in the aqueous phase. Separate, wash the light petroleum solution twice with water, and evaporate the extract by heating to not more than 30° C under reduced pressure or in a current of nitrogen. Dissolve the residue in 1 ml of light petroleum

(boiling range 60° to 80° C). This solution should if possible contain at least 250 μg of the nitroso-tocopherol per ml.

Preparation of papers—Use Whatman No. 1 filter-paper (for chromatography). Cut the paper into strips, 20 cm × 3 cm. (If the proportion of one nitroso-tocopherol to the other is high, wider strips—up to 6 cm—may be used.) Draw, in pencil, a starting line 2 cm from one of the edges, and dip the paper in the liquid paraffin solution from the other edge, until the strip is immersed to within 1 cm of the pencil line. Remove from the solution, and allow the solvent to evaporate. When small amounts of nitroso-tocopherols are to be chromatographed (about 5 μg), the papers should first be impregnated with zinc carbonate solution containing 5 to 10 p.p.m. of sodium fluorescein,[5] so that the nitroso-derivatives can be seen clearly in ultra-violet light.

Chromatographic separation—Add the solution in light petroleum to the starting line of the paper from a glass micropipette (a suitable type of disposable pipette can be made by drawing an ordinary-melting-point tube to a tip and calibrating to a mark by weight of water). Add the solution as a narrow band across the whole width of the paper. It is recommended that 50 μg of each nitroso-derivative be added, if this amount is available, although as little as 5 μg can be used, with some corresponding loss of accuracy. Hang the test papers and a blank strip side by side in a chromatography tank so that the lower edges just dip in the developing solvent, which should normally be 93 per cent. v/v ethanol in water; under certain conditions of humidity, 95 per cent. v/v ethanol may give more rapid separation. Develop the papers for 2 to 3 hours, remove them from the tank and dry them in a gentle current of air. Cut out the yellow bands (the dark bands in ultra-violet light), roll the strips of paper into cylinders, and elute each separately with 3 ml of ethanol in a small tube, 10 cm × 1·5 cm, fitted with a B14 stopper.

Determination—Determine the nitroso-tocopherols by the modified Emmerie - Engel method, as previously described,[3,4,5] but measure the extinctions of both the test and blank solutions after 60 minutes. The determination should if possible be carried out in a darkened room under artificial light. During the 60-minute oxidation period, the tubes must be placed in total darkness.

To calculate the results, either use the given factors or establish calibration curves by the separate nitrosation of 500 μg of each tocopherol. Chromatograph and determine in 10 to 100-μg stages.

RESULTS

ANALYSIS OF SYNTHETIC MIXTURES—

Table VI shows the results for several mixtures of synthetic β- and γ-tocopherol and one mixture of ϵ- and η-tocopherol.

Although at least 5 μg of each nitroso-derivative were needed for quantitative analysis, as little as 0·2 μg could be easily detected on paper impregnated with zinc carbonate rendered fluorescent by the addition of sodium fluorescein. Some experiments were carried out to determine the minimum amount of β-tocopherol that could be positively detected in admixture with 100 μg of γ-tocopherol. It was found that the detection of less than about 1 μg of

TABLE VI

SEPARATION OF MIXED TOCOPHEROLS

Mixture	Amount of each tocopherol taken, μg	Amount of each tocopherol found, μg
β- and γ-tocopherol	25·5 β, 82·0 γ	24·5 β, 83·0 γ
β- and γ-tocopherol	13·0 β, 100·0 γ	12·8 β, 98·2 γ
β- and γ-tocopherol	52·0 β, 100·0 γ	51·8 β, 99·2 γ
ϵ- and η-tocopherol	24·6 ϵ, 28·8 η	23·2 ϵ, 27·6 η

β-tocopherol was complicated by the presence of an impurity produced during nitrosation. The substance responsible was observed as a dark shadow in ultra-violet light, close to the position normally occupied by β-tocopherol. It appeared to arise partly from the reagents used and partly from the purest specimens of both synthetic and natural γ-tocopherol. Attempts to eliminate the material were unsuccessful, and experiments on the detection of sub-microgram amounts of β-tocopherol were discontinued. It was important, though, in the analysis of the oils described below, to ensure that the level of β-tocopherol observed and

measured was at least 1 μg; this means that at least 100 μg of the β - γ mixture had to be taken to detect positively 1 per cent. of β-tocopherol.

THE DETERMINATION OF β- AND ε-TOCOPHEROL IN NATURAL OILS—

Maize, rice and cottonseed oils were examined. Maize oil was extracted from fresh seed, rice oil was extracted from commercial unpolished rice and cottonseed oil was a crude commercial sample.

Maize oil—Two grams were saponified, and the unsaponifiable fraction was purified by chromatography on a number of sheets of paper by the two-dimensional method. From each paper the isolated spots containing only β- and γ-tocopherol were removed, and the tocopherols were eluted with ethanol. The solution was evaporated to dryness and the residue was dissolved in a little benzene. The total solution was spotted on to wide sheets of plain Whatman No. 1 filter-paper, and the material was chromatographed with methanol as mobile phase. The tocopherols migrated with the solvent front, leaving traces of liquid paraffin behind. The tocopherol fraction was eluted with light petroleum. A solution of 150 μg of the purified mixed tocopherols was nitrosated by the proposed method, and the β-tocopherol was determined. It was found that 9·1 per cent. of the γ-tocopherol spot was β-tocopherol.

Rice oil—Eighteen grams of rice oil were saponified and purified by floridin earth chromatography. The purified extract was chromatographed on wide sheets of Whatman No. 1 filter-paper, with methanol as mobile phase, to separate the tocopherols from much inert material. The tocopherol fraction was located near the solvent front and was eluted with light petroleum. The material was then re-chromatographed on paper impregnated with zinc carbonate and the η-tocopherol band isolated and eluted with light petroleum. The tocopherol (113 μg) was dissolved in 5 ml of ethanol and nitrosated. No ε-tocopherol was found in the η-tocopherol fraction.

Cottonseed oil—Four grams were saponified and purified by floridin earth chromatography. The purified material was dissolved in 1 ml of benzene and chromatographed on wide strips of paper impregnated with zinc carbonate. The γ-tocopherol band separated easily and 346 μg (by assay) were eluted and dissolved in 5 ml of ethanol. The total amount was nitrosated and chromatographed as described under "Method." The γ-tocopherol fraction was found to contain 0·4 per cent. of β-tocopherol.

REFERENCES

1. Green, J., and Marcinkiewicz, S., *Analyst*, 1959, **84**, 297.
2. Quaife, M. L., *J. Biol. Chem.*, 1948, **175**, 605.
3. Eggitt, P. W. R., and Ward, L. D., *J. Sci. Food Agric.*, 1953, **4**, 569.
4. Green, J., Marcinkiewicz, S., and Watt, P. R., *Ibid.*, 1955, **6**, 274.
5. Green, J., *Ibid.*, 1958, 9, 801.

NOTE—Reference 1 is to Part I of this series.

The Determination of Thorium in Ores by Liquid-Liquid Extraction

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A rapid method is described for the determination of thorium in medium-grade ores. Thorium is separated from gross amounts of other elements by extraction from nitric acid to which aluminium nitrate has been added as a salting-out agent, with *iso*butyl methyl ketone containing 10 per cent. v/v of tri-*n*-butyl phosphate. The aqueous layer contains *meso*tartaric acid, which forms a complex with any zirconium present and prevents its extraction. Thorium is extracted from the organic phase with water and is finally determined absorptiometrically with 1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid.

IN recent years, the increased interest in the uses of thorium has led to a demand for more rapid and more certain methods for determining this metal in its ores. Classical methods for

the separation of thorium have now been partly superseded by more rapid procedures, *e.g.,* those depending on chromatography on cellulose.[1] This paper describes a solvent-extraction procedure for determining thorium, which is considered to have as great a precision as, and to be more rapid than, other procedures in current use.

The first study of a solvent-extraction procedure for thorium was made by Levine and Grimaldi,[2] who used a large number of oxygen-containing organic solvents to investigate the extraction of thorium from thorium nitrate solutions containing aluminium nitrate as salting-out agent. They chose mesityl oxide as being the most satisfactory solvent, and it has since been used by other workers.[3,4] However, these methods suffer from the disadvantage that aerial oxidation turns the solvent brown; oxidation is rapid in extraction systems containing nitric acid.

It is well known that tri-*n*-butyl phosphate is a good extractant for thorium from nitric acid,[5] and moreover, it is resistant to oxidation.[6] Thorium can be removed from nitric acid by continuous extraction with a 40 per cent. solution of tri-*n*-butyl phosphate in xylene, and such a procedure has been used to determine thorium.[7] We have used tri-*n*-butyl phosphate as extractant and *iso*butyl methyl ketone (hexone) as diluent. Dilution of the tri-*n*-butyl phosphate considerably reduces the extraction of rare earths. Aluminium nitrate is used as salting-out agent and permits the thorium to be quantitatively removed by a single extraction. After extraction, thorium is stripped from the organic phase with water and is then determined absorptiometrically with 1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid (APANS). APANS has been widely used as a selective reagent for thorium,[3,4,8,9,10] and zirconium is one of the few metals that interfere appreciably. To overcome this interference, *meso*tartaric acid is initially added to the aqueous phase; this forms a complex with zirconium and prevents its extraction by the tri-*n*-butyl phosphate - hexone solvent. *meso*Tartaric acid has been used as a complexing agent for zirconium in other methods of determining thorium.[11,12]

The extraction procedure has been successfully applied to the determination of thorium in medium-grade ores, *e.g.,* monazites, thorites, fergusonite and black sands. Low-grade ores (containing less than 1 per cent. of thoria) have not been examined; for these ores, the success of the method would depend on the nature of the preponderant elements.

EXPERIMENTAL

SOLVENT-EXTRACTION SYSTEM—

A 10 per cent. v/v solution of tri-*n*-butyl phosphate in hexone was found to be a satisfactory extractant. Thorium is quantitatively removed by one extraction with 20 ml of this solvent from a solution containing 1 mg of ThO_2, as nitrate, in 10 ml of 15 per cent. v/v nitric acid, to which 19 g of aluminium nitrate are added. In addition to its salting-out properties, aluminium nitrate has the advantage that it forms a complex with phosphate, which would otherwise tend to interfere with the extraction of thorium.[2]

Some complexing agents were tested for their ability to retain zirconium selectively in the aqueous phase, and this work is described in detail elsewhere.[13] *meso*Tartaric acid was found to be satisfactory. The thorium loss in the general procedure (see p. 314) was 0·4 per cent. in the extraction and 0·003 per cent. in the stripping step (in this experiment and in the general development of the method, thorium-228 was used as tracer[13]). The total percentage loss is small and does not affect the accuracy of the method, in which standard thorium nitrate solution is extracted under identical conditions for comparison with the sample.

When present in large amounts, zirconium forms a gelatinous precipitate with *meso*tartaric acid when the solution is warmed to dissolve aluminium nitrate and *meso*tartaric acid. Some thorium is entrained in this precipitate, and it is therefore necessary to modify the procedure in order to determine thorium in thorites, which contain a considerable amount of zirconium. In the modified procedure, *meso*tartaric acid solution is added in the cold to a solution of the other reagents. Under these conditions, formation of the precipitate is delayed and extraction can be carried out before precipitation begins.

ABSORPTIOMETRIC DETERMINATION OF EXTRACTED THORIUM—

Initially, attempts were made to determine thorium directly, without the stripping step, by adding APANS solution and a sufficient volume of ethanol to an aliquot taken from the organic layer. Optical-density values were not sufficiently reproducible, possibly owing to slight oxidation of the solvent by nitric acid. Such a procedure would only be suitable for rapid semi-quantitative determinations of thorium, *e.g.,* in geochemical prospecting. For

quantitative work, it is necessary to strip the thorium from the organic layer; this can be done by extraction with water.

The thorium in the aqueous extract is determined absorptiometrically with APANS. Calibration curves are slightly convex to the concentration axis, and, since they cannot be exactly reproduced from day to day, it is necessary to prepare standards (duplicates, each containing $1 \cdot 2$ mg of ThO_2) simultaneously with the determination of thorium in a solution of unknown concentration. The optimum concentration of thorium in the final solution is $0 \cdot 4$ to $0 \cdot 8$ mg of ThO_2 per 100 ml, and, in this range, the degree of departure from Beer's law is only of the order of 1 per cent. It is therefore permissible to calculate the apparent thorium content of a sample by means of the following expression—

$$\text{Thorium present, as } ThO_2, \text{ mg} = \frac{\text{Optical density of sample solution} \times 1 \cdot 2}{\text{Average optical density of } 1 \cdot 2\text{-mg } ThO_2 \text{ standard solutions}}$$

For concentrations below $0 \cdot 4$ mg of ThO_2 per 100 ml, optical-density values are less reproducible, and, at concentrations above $0 \cdot 8$ mg of ThO_2 per 100 ml, the curvature of the calibration graph increases considerably.

METHOD

REAGENTS—

Potassium hydroxide—Analytical-reagent grade.
Nitric acid, concentrated.
Hydrofluoric acid, 25 *per cent. v/v.*
Ammonia solution, sp. gr. 0·880.
Aluminium nitrate—Hydrated aluminium nitrate, $Al(NO_3)_3.9H_2O$ was used.
meso*Tartaric acid monohydrate.*
Standard thorium solution—Prepare a solution of Specpure thorium nitrate, $Th(NO_3)_4.4H_2O$, containing $1 \cdot 2$ mg of ThO_2 per 3 ml.
Extraction mixture—Mix 1 volume of tri-*n*-butyl phosphate, purified by washing with 1 per cent. sodium carbonate solution and then with water, and 9 volumes of redistilled *iso*butyl methyl ketone.
Hydrochloric acid, concentrated.
APANS solution—Prepare a 0·2 per cent. w/v aqueous solution of the sodium salt of 1-(*o*-arsonophenylazo)-2-naphthol-3 : 6-disulphonic acid.
Ethanol, absolute.
Perchloric acid, 60 per cent.
meso*Tartaric acid solution*—Prepare a 20 per cent. w/v aqueous solution of *meso*tartaric acid monohydrate.

GENERAL PROCEDURE—

Weigh 10 g of potassium hydroxide pellets into a nickel crucible 5 cm in diameter and 5 cm deep, and heat to redness to remove water. Allow to cool, and add an accurately weighed 1-g sample of ore. Cover the crucible, heat slowly to redness, and continue to heat for 1 hour. Allow to cool, and leach the melt with the minimum volume of dilute nitric acid in a 500-ml beaker covered with a clock-glass. Wash the crucible with concentrated nitric acid and then with water, and add the washings to the solution in the beaker. Make the solution 25 per cent. v/v with respect to nitric acid, and bring to the boiling-point. Add 1 drop of 25 per cent. hydrofluoric acid, and boil until the melt is completely broken down. Allow to cool, and add ammonia solution, sp.gr. 0·880, until the solution is almost neutral. Heat to the boiling-point, cool slightly, and neutralise with ammonia solution. Add a slight excess of ammonia solution. Collect the precipitate on a Whatman No. 541 filter-paper, and wash twice with hot water containing a few drops of ammonia solution. Open the paper over the original beaker, and wash the precipitate into the beaker with a jet of hot water. Complete the washing with a measured volume of nitric acid (sufficient to give an acidity of 15 per cent. v/v with respect to nitric acid when the solution is subsequently diluted to a fixed volume). Cover the beaker with a clock-glass, and gently boil the solution for about 15 minutes. Thoroughly cool, and transfer the solution to a calibrated flask of suitable volume (a 10-ml aliquot should contain 0·8 to 1·6 mg of ThO_2). Also transfer, by means of a rubber-covered glass rod, any insoluble solid matter, as this may retain thorium-containing solution. Allow to cool, dilute to the mark with water, and shake thoroughly.

Weigh 19 g of aluminium nitrate and 0·2 g of *meso*tartaric acid into each of a number of dry 60 to 70-ml extraction tubes fitted with B24 ground-glass joints and "open" stoppers. In the first tube, A, place 8·5 ml of water and 1·5 ml of concentrated nitric acid. In the second and third tubes, B and C, place 5·5 ml of water, 1·5 ml of concentrated nitric acid and, by means of a 10-ml graduated burette, 3 ml of standard thorium solution. In the remaining tubes, place, by pipette, 10-ml portions of the solutions prepared from the thorium-containing ore samples. Warm the tubes in hot water, and swirl them gently to dissolve the solid reagents. Avoid wetting the necks of the tubes with the solutions. Cool thoroughly, and add, by pipette, 20 ml of extraction mixture to the contents of each tube. Vigorously shake each tube for 1 minute, and set aside for 1 hour. By pipette, carefully transfer a 10-ml portion of each organic layer to a 50-ml Pyrex-glass separating funnel fitted with a B19 "Grip Seal" stopper and joint. Shake each organic solution successively with 20, 20 and 10-ml portions of water, and collect the combined aqueous extracts in 100-ml calibrated flasks.

To the contents of each flask, add, in this order, 2 ml of concentrated hydrochloric acid, 5 ml of APANS solution and 20 ml of absolute ethanol. Shake to mix the components, allow to cool for 20 minutes, dilute to the mark with water, and thoroughly mix. Set the solutions aside for 45 minutes, and then measure the optical densities at 550 mμ with a Unicam SP500 spectrophotometer (red photocell). Use the solution derived from the extraction in tube A as blank and those derived from the extractions in tubes B and C as thorium standards.

Derive the amount of thorium present in the sample solutions by means of the expression on p. 314.

Procedure for thorites—

Carry out the procedure described above as far as the precipitation with ammonia solution. Wash the hydroxide precipitate back into the original beaker with water and nitric acid (the volume of nitric acid used is not critical). Cover the beaker with a clock-glass, and evaporate the solution to a small volume. Remove the clock-glass, and evaporate the solution to dryness under an infra-red lamp. Add 10 to 15 ml of 60 per cent. perchloric acid, and evaporate to dryness under an infra-red lamp to fix the silica in the sample. Add sufficient nitric acid to give an acidity of 15 per cent. v/v with respect to this acid when the solution is subsequently diluted to a fixed volume, cover with a clock-glass, and warm on a steam-bath for about 15 minutes. Thoroughly cool, and transfer the total contents of the beaker (see "General Procedure") to a calibrated flask of suitable volume (a 5-ml aliquot should contain 0·8 to 1·6 mg of ThO$_2$). Dilute to the mark with water, and shake thoroughly.

TABLE I

EFFECT OF VARIOUS IONS ON GENERAL PROCEDURE

Ion	Amount of ion added, mg	Error of optical-density value, %
Cu^{2+}	20	+0·1
Co^{2+}	20	−0·1
Ni^{2+}	20	−0·1
Zn^{2+}	20	+0·7
Pb^{2+}	20	−0·4
UO$_2$$^{2+}$	5 (U)	+0·9
Fe^{3+}	20	+4·1
La^{3+}	20	+0·9
Ce^{3+}	20	−0·1
Ce^{4+}	20	+1·7
Y^{3+}	20	+0·7
Zr^{4+}	25 (ZrO$_2$)	−0·9*
SO$_4$$^{2-}$	100	−0·1
PO$_4$$^{3-}$	20	−0·7
B$_4$O$_7$$^{2-}$	10 (B)	+0·1

* Procedure for analysing thorites was used.

Weigh 19 g of aluminium nitrate into each of the required number of dry extraction tubes. In the first tube, A, place 7·5 ml of water and 1·5 ml of concentrated nitric acid. In the second and third tubes, B and C, place 4·5 ml of water, 1·5 ml of concentrated nitric acid and, by means of a graduated burette, 3 ml of standard thorium solution. In each of the

remaining tubes, place, by pipette, a 5-ml portion of the solution prepared from the thorium-containing ore sample, 3·25 ml of water and 0·75 ml of concentrated nitric acid. Dissolve the solid reagent as before, and thoroughly cool the tubes. By pipette, add 1 ml of *meso*-tartaric acid solution to the contents of each tube, swirl carefully to mix the solutions, and add 20 ml of extraction mixture. Continue as described under "General Procedure."

INTERFERENCE

The degree of interference, if any, of several common cations and anions with the proposed general procedure was determined by adding the individual ions to solutions containing thorium equivalent to 1·2 mg of ThO_2. Table I shows the percentage error caused by these ions in the optical-density values found for thorium.

RESULTS

Some thorium-containing ores were analysed by the proposed and alternative methods; the results are shown in Table II.

TABLE II

COMPARISON OF RESULTS BY THE PROPOSED AND ALTERNATIVE METHODS

Sample	Amount of thorium found by solvent extraction, %	Amount of thorium found by alternative method, %	Alternative method used
Indian monazite	10·05	{ 9·95	See Williams, A. F.[1]
		10·05	See Clinch, J., et al.[14]
South African monazite	6·05	{ 6·05	See Williams, A. F.[1]
		6·10	See Clinch, J., et al.[14]
Norwegian monazite	12·30	12·15	Chromatography on cellulose and then hydroxide precipitation
Black sand	3·20	3·20	} Solvent extraction of a spiked sample
Fergusonite	3·40	3·45	} sample
Monazite concentrate	3·05	3·10	
Swaziland monazite	4·75	4·80	
Monazite	5·55	5·70	} See Williams, A. F.[1]
Monazite	6·10	6·20	
Thorite	6·20	6·40	
Thorite	5·25	5·35	See Williams, A. F.[1]
		5·20	Two chromatographic separations on cellulose and then oxalate precipitation
		5·25	Two iodate and then two oxalate precipitations
Thorite	6·80	{ 6·85	See Williams, A. F.[1]
		6·85	See Everest, D. A., et al.[12]
Thorite residue	0·25	0·26	See Everest, D. A., et al.[12]
Barytes - thoria*	0·98	—	—
Galena - thoria†	0·98	—	—

* Radiometric standard containing 1 per cent. of thorium, as ThO_2.
† Radiometric standard containing 1 per cent. of thorium, as ThO_2; thorium was first separated from lead by oxalate precipitation.

REFERENCES

1. Williams, A. F., *Analyst*, 1952, **77**, 297.
2. Levine, H., and Grimaldi, F. S., United States Atomic Energy Commission Report AECD-3186, 1950.
3. Banks, C. V., and Byrd, C. H., *Anal. Chem.*, 1953, **25**, 416.
4. Cuttitta, F., United States Department of Interior, Geological Survey, Trace Elements Investigation, Report No. 498, 1955.
5. Hesford, E., McKay, H. A. C., and Scargill, D., *J. Inorg. Nuclear Chem.*, 1957, **4**, 321.

6. Warf, J. C., *J. Amer. Chem. Soc.*, 1949, **71**, 3257.
7. Laboratory Manual Method No. 437, United Kingdom Atomic Energy Authority, Chemical Services Department, Springfields, 1956.
8. Thomason, P. F., Perry, M. A., and Byerly, W. M., *Anal. Chem.*, 1949, **21**, 1239.
9. Mayer, A., and Bradshaw, G., *Analyst*, 1952, **77**, 154.
10. Clinch, J., *Anal. Chim. Acta*, 1956, **14**, 162.
11. Fletcher, M. H., Grimaldi, F. S., and Jenkins, L. B., *Anal. Chem.*, 1957, **29**, 963.
12. Everest, D. A., and Martin, J. V., *Analyst*, 1957, **82**, 807.
13. Martin, J. V., Scientific Report CRL/AE164.
14. Clinch, J., and Simpson, E. A., *Analyst*, 1957, **82**, 258.

A Versatile Electronic Device for Counting Drops of Eluant in Chromatography or Operating Ancillary Apparatus after Pre-set Counts or Pre-set Times

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An electronic device in which three selector dekatrons are used has been constructed, primarily to count drops of effluent falling from an ion-exchange column and to operate external equipment, *e.g.*, a sample-changer, after a pre-selected number between 1 and 1000 has been counted at rates up to 3 drops per second. The apparatus can be operated by closing a pair of contacts, by positive and negative impulses or by the interruption of a beam of light. It is especially suitable for the collection of small sample volumes, but the actual volume can be varied at will between about 0·05 and 50 ml even during elution. The apparatus can also be used as an interval-timer to operate an external circuit at regular intervals within the range 1 to 20 seconds, 1 to 500 seconds or 1 to 500 minutes.

IN studies of the separation of small amounts of certain metals by ion exchange, it was necessary to collect successive fractions, the volume of which never exceeded 2 ml and was often as small as 0·2 ml. Commercial sample-changers were not readily adaptable to this task and it was decided to construct an apparatus that would control the operation of sample-changing after a pre-selected number of drops of effluent had been counted and would then automatically re-set its counting circuits. A further refinement was to provide for changes in the volume of sample collected at any stage in the elution. The final design incorporates a number of novel features that greatly extend its useful range of applications.

CIRCUIT—

The circuit is shown in Fig. 1 and is an elaboration of that described by Snow, Porter and Tomalin.[1] The properties of selector cold-cathode dekatrons are used as counting devices. Each dekatron, V_3, V_5 or V_7, contains a central anode connected to a 450-volt line and has ten cathodes, K_0, K_1, K_2, . . . K_9 evenly spaced around the circumference of a circle and normally held at earth potential. Adjacent to each of these cathodes is another electrode; these ten secondary electrodes are internally connected and constitute the first guide electrode, G_1. Another set of ten internally connected electrodes adjacent to the first set constitute the second guide electrode, G_2. When read clockwise, the order of electrodes is—K_0, G_1, G_2, K_1, G_1, G_2 . . . K_9, G_1 and G_2. Guides G_1 and G_2 are normally held at +36 volts above earth potential, and a red glow rests on one of the cathodes.

DEKATRON DRIVE CIRCUITS—

Each dekatron is associated with a small cold-cathode thyratron, V_2, V_4 or V_6. The trigger electrode is normally held at +165 volts, but the tube will fire when a positive pulse of at least 10 volts is applied. In practice, a pulse of about +45 volts is applied. However, the total resistance in the anode circuit (458,000 ohms) is so large that, when fired, the voltage drop will reduce the anode potential below that required for maintaining the discharge, so that this is immediately quenched. A short sharp negative pulse is thus produced.

K_8 is a small subsidiary cathode connected to a −100-volt line through a 10-megohm

resistor. This keeps a small discharge alight between the anode and K_8, so that the gas is kept ready ionised for the main discharge to fire.

OPERATION OF SELECTOR DEKATRONS—

The pulse from the thyratron is fed to guide G_1 on the dekatron through a 1000-$\mu\mu$F condenser, C_{22}, C_{24} or C_{26}, and to guide G_2 through a 2000-$\mu\mu$F condenser, C_{21}, C_{23} or C_{25}. There is thus a brief time-lag between its arrivals at G_1 and G_2. When the pulse arrives at G_1, the discharge will be transferred from one of the cathodes K_0, K_1, etc. to the adjacent electrode connected to G_1, which is now, momentarily, more negative than the cathodes. As this guide returns to its normal potential of $+36$ volts, the more delayed negative pulse arrives on G_2 and the discharge is transferred to the adjoining electrode connected to G_2. Finally, as G_2 in turn returns to its normal potential, the discharge moves on to the next cathode and the red glow observed at the front of the instrument panel against an engraved scale shows an increase of one in serial number between 0 and 9, inclusive.

PROVISION FOR PRE-SELECTED COUNTS—

In a conventional dekatron scaler, the circuits are so arranged that each tenth impulse returns the discharge to K_0 and simultaneously supplies a signal pulse to the succeeding dekatron, which records each group of ten pulses from the preceding stage as a single event. In our instrument, this sequence is greatly modified by the provision of three switches, S_1, S_2 and S_3, which act as "count selectors." S_1 and S_2 are identical four-position four-bank switches connected, as shown in Fig. 1, to the cathodes of V_3 and V_5, respectively. In each instance, cathode K_0 is connected to earth and K_a, K_b and K_c are connected to the "reset line." K_a is connected to cathodes K_2, K_4, K_6 and K_8. K_b is connected only to K_5. K_c is connected to cathodes K_1, K_3, K_7 and K_9. When read clockwise, the switch positions are labelled (*i*) "Stage out," (*ii*) $\times$ 2, (*iii*) $\times$ 5, and (*iv*) $\times$ 10, since, in these positions, a signal pulse is transmitted to the following dekatron after 1, 2, 5 or 10 pulses have been received. The third switch, S_3, is a ten-position selector connected severally through 100,000-ohm resistors, R_{48} to R_{57}, and 0·01-μF condensors, C_7 to C_{16}, to cathodes K_0 to K_9 of dekatron V_7. (In the interests of clarity, only the connections to K_1, K_8, K_9 and K_0 are shown in Fig. 1.) With various settings of this switch it is possible to count any number from 1 to 10 before a signal is transferred to the next (and output) stage. The total pre-selected count is obtained by multiplying the number on the last selector, S_3, by the factors shown on the other two, counting "Stage out" as $\times$ 1. The number 160 is thus obtained by setting $\times$ 10, $\times$ 2 and 8, and the number 9 is obtained by setting "Stage out," "Stage out" and 9. Each selector switch refers to the dekatron situated immediately below it on the instrument panel, and the following pre-set counts can be obtained—

    1, 2, 3, 4, 5, 6, 7, 8, 9.
    10, 12, 14, 15, 16, 18.
    20, 24, 25, 28.
    30, 32, 35, 36.
    40, 45.
    50.
    60.
    70, 75.
    80.
    90.
    100, 120, 125, 140, 150, 160, 175, 180.
    200, 225, 250.
    300, 350.
    400, 450.
    500, 600, 700, 800, 900, 1000.

INPUT STAGE—

The grid of pentode V_1 is biased at -6 volts and in its normal state is conducting moderately. Contact A is normally at $+100$ volts. When contacts A and B are shorted, the potential falls to earth, so that a large negative impulse is fed on to the valve grid through condenser C_{18}. The valve is thus driven to cut-off and the potential at its anode rises to $+300$ volts. A positive pulse is thus produced and is fed through C_{19} to the trigger stages (as selected by S_1 and S_2) and thence to one of the dekatrons. Since the pulse produced is somewhat large,

it is divided by C_{19} and C_{20}. Contacts A and B are most conveniently shorted by allowing each drop of effluent on its way to the collecting vessel to fall between a pair of platinum electrodes in close proximity. For other purposes, it may be preferable to count drops (or, indeed, any other objects) by momentarily interrupting a beam of light falling on a photocell.

In addition to being triggered by a positive pulse obtained as described above, the counting device can be triggered by a positive pulse of not less than 45 volts applied through condenser C_{32} (contact D) or by a negative pulse of not less than 15 volts applied through condenser C_{17} (contact C). Provision for internal testing can be made by connecting the negative input, contact C, to the -90-volt tap on the transformer.

OUTPUT STAGE—

Valve V_8 receives a positive pulse from one of the cathodes of dekatron V_7 and conducts heavily. Relay REL/4 in the anode circuit closes and operates the change-over contacts REL.B. This causes C_{31} to discharge on to the valve grid and thus delays the release of the relay for approximately 0·3 second, which allows ample time for the operation of a sample-changer before counting is resumed. For drop-counting, the rate must not therefore exceed 2 to 3 drops per second. Provision for shorter or longer delays can readily be made by altering the values of R_{35} and C_{31}.

Simultaneously with the change-over of REL.B, contact REL.A opens, whereby the potential of the reset line is raised to about $+100$ volts. Since all the cathodes of the dekatrons are connected to this line, with the exception of the three cathodes designated K_0, the discharge immediately moves to these cathodes and the assembly is ready to begin another count. The application of this voltage to the cathodes of the thyratrons also prevents them from operating on the carry pulse produced on the zero cathodes of the dekatrons when the discharge returns to those cathodes.

Further contacts on REL/4, which are operated by pulses reaching the output stage, are a make-switch, REL.C (contacts E and F), and a change-over switch, REL.D (contacts G, H, and I); these are available for operating subsidiary apparatus, such as a sample-changer or an electro-mechanical register, or both.

SUBSIDIARY CIRCUITS—

A mains transformer and half-wave metal rectifiers provide, with the help of stabilising valves V_9 and V_{10} and a resistor network, the requisite stabilised voltages of $+300$, $+165$ and $+36$ volts, as well as unstabilised voltages of $+450$, -6 and -100 volts. A separate winding provides 6·3 volts a.c. for the indirectly heated cathodes of V_1 and V_8 and for the pilot light.

RESET SWITCH—

S_4 is a spring-loaded switch that is normally closed. When depressed, it opens the circuit and allows about $+100$ volts to be placed on the reset line. *This switch must only be operated momentarily; it must not be held depressed.*

COUNT SWITCH—

S_6 is a single-pole single-throw switch, which, in the up-position, disconnects the input circuit so that counting is suspended.

CONTINUOUS COUNT SWITCH—

S_7 is a single-pole, single-throw switch. When closed, it prevents the application of a positive voltage to the reset line, even when REL/4 operates and causes the contacts of REL.A to open. Counting will thus continue without interruption even although an external piece of equipment has been operated by REL.C or REL.D after a certain number of counts and will continue to be activated at regular intervals. It is, however, important to note that, if this facility is being used, the selector switch on the last stage, S_3, must be set to 10. The number pre-selected is thus restricted to 10, 20, 40, 50, 100, 200, 250, 500 or 1000.

USE AS AN INTERVAL TIMER—

By setting selector switches S_1, S_2 and S_3 to some pre-arranged number and connecting a timing unit (e.g., an Air Ministry surplus timing unit, Contact Master Type 2, which closes a circuit every 0·5 second and every 0·5 minute) between A and B, the assembly can be made to operate an external circuit through REL.C or REL.D at regular intervals up to 500 seconds or 500 minutes.

PRE-SET COUNT AND STOP—

With S_7 closed to prevent automatic re-setting and the double-pole double-throw switch S_8 in the position shown in Fig. 1, REL.C is disconnected from contacts E and F and the relay coil is connected to the reset line through REL.C and resistor R_{58}. When a pre-selected count has been reached, the closing of REL/4 will cause REL.C to close and so lock in the relay. Re-setting is accomplished by operating reset switch, S_4, which reduces the current through the relay to a value below that needed for its holding.

EXTERNAL CONNECTIONS—

Connections A to I inclusive (see Fig. 1) are brought out to a terminal strip at the rear of the instrument. Connections A to C are used for various types of input signal; connections E to I go to the contacts of relay REL/4 and provide a number of switching possibilities for external circuits, e.g., to control the turntable of a fraction-collector, which will be actuated after a pre-set number of input pulses.

APPENDIX

LIST OF COMPONENTS

The mains input plug was a Bulgin 3-pin shrouded plug.

V_1, V_8	=	Mullard EF91 pentode.
V_3, V_4, V_6	=	Ericcson GTE175M thyratron.
V_5, V_5, V_7	=	Ericcson GS10C selector dekatron.
V_9, V_{10}	=	Ericcson GD150M/S voltage-stabiliser.
MR_1, MR_2	=	STC Type RM4 half-wave metal rectifier.
MR_3	=	STC Type RM1 half-wave metal rectifier.
S_1, S_2	=	Single-pole four-way four-bank.
S_3	=	Single-pole ten-way.
S_4	=	Re-set (push to break).
S_5	=	Test pulse (push to make).
S_6, S_7	=	Single-pole single-throw.
S_8	=	Double-pole double-throw.
REL/4	=	5000-ohm coil relay; one make, one break and two change-over contacts.
T	=	Mains transformer: +425,0, −90 volts and 6·3 volts. Rating: +425 volts, 100 mA; −90 volts, 25 mA; 6·3 volts, 4 amperes.
R_1	=	300,000 ohm.
R_2	=	680,000 ohm.
R_3	=	2·2 megohm.
R_4	=	1000 ohm.
R_5-R_9, R_{10}, R_{15}, R_{16}, R_{21}, R_{22}, R_{27}, R_{30}, R_{39}, R_{40} to R_{57}	=	100,000 ohm.
R_6, R_{12}, R_{18}, R_{26}, R_{33}	=	1 megohm.
R_7, R_{13}, R_{19}	=	390,000 ohm.
R_8, R_{14}, R_{20}	=	68,000 ohm.
R_{11}, R_{17}, R_{23}	=	560,000 ohm.
R_{24}, R_{38}	=	4700 ohm.
R_{25}	=	15,000 ohm, 5 watt.
R_{28}	=	220,000 ohm.
R_{29}	=	25,000 ohm.
R_{31}, R_{32}	=	10,000 ohm, 5 watt.
R_{34}	=	1·5 megohm.
R_{35}, R_{36}, R_{37}	=	10 megohm.
R_{58}	=	100,000 to 130,000 ohm (adjust to suit relay characteristics).
P_1	=	250,000-ohm wire-wound potentiometer; tapping at +165 volts must be set by using a valve voltmeter.
C_1 to C_{18}, C_{20}, C_{32}	=	0·01 μF.
C_{19}	=	0·005 μF.
C_{21}, C_{23}, C_{25}	=	2000 $\mu\mu$F ± 5 per cent.
C_{22}, C_{24}, C_{26}	=	1000 $\mu\mu$F ± 5 per cent.
C_{27}, C_{28}, C_{29}, C_{30}	=	8 μF electrolytic, 1000-volt working.
C_{31}	=	0·1 μF.

NOTE—Unless otherwise stated, all resistors are 0·5-watt ± 10 per cent. and all condensers are 400-volt working.

REFERENCE

1.   Snow, A., Porter, F. A. E., and Tomalin, A. W., *J. Sci. Instrum.*, 1958, **35**, 243.

+36V   R11   +36V   R17

R9
R10
R7
C21   G2   V3   Ko Ka Kb Kc
R8
C22   GI

R15
R16
R13
C23   G2   V5   Ko Ka Kb Kc
R14
C24   GI

R19

R20

C32

+ve ▼ D
pulse
INPUT

−ve ▲ C
pulse

+165 V
R6

+165 V
R12

+165 V
R18

R5

+150
V

R1

C18

C17

R4

V1

V2   Ks

R40
to
R43

V4   Ks

R44
to
R47

6·3V
a.c.

C19

S6

C1

C4

R3

−6V

C20

R35

C2

C5

R2

R36

C3

C6

B
S5

S1

S2

Contact input
Negative pulse
Positive pulse
Relay C
Relay D

A B C D E F G H I

B7G
h   A
K   G3
GI   G2
V1, V8

B7G
K
T   Ks
A
V2, V4, V6

B7G
K   A
K
K   K
A   K
V9, V10
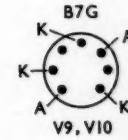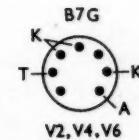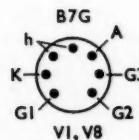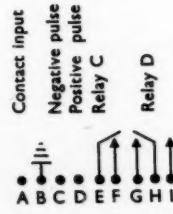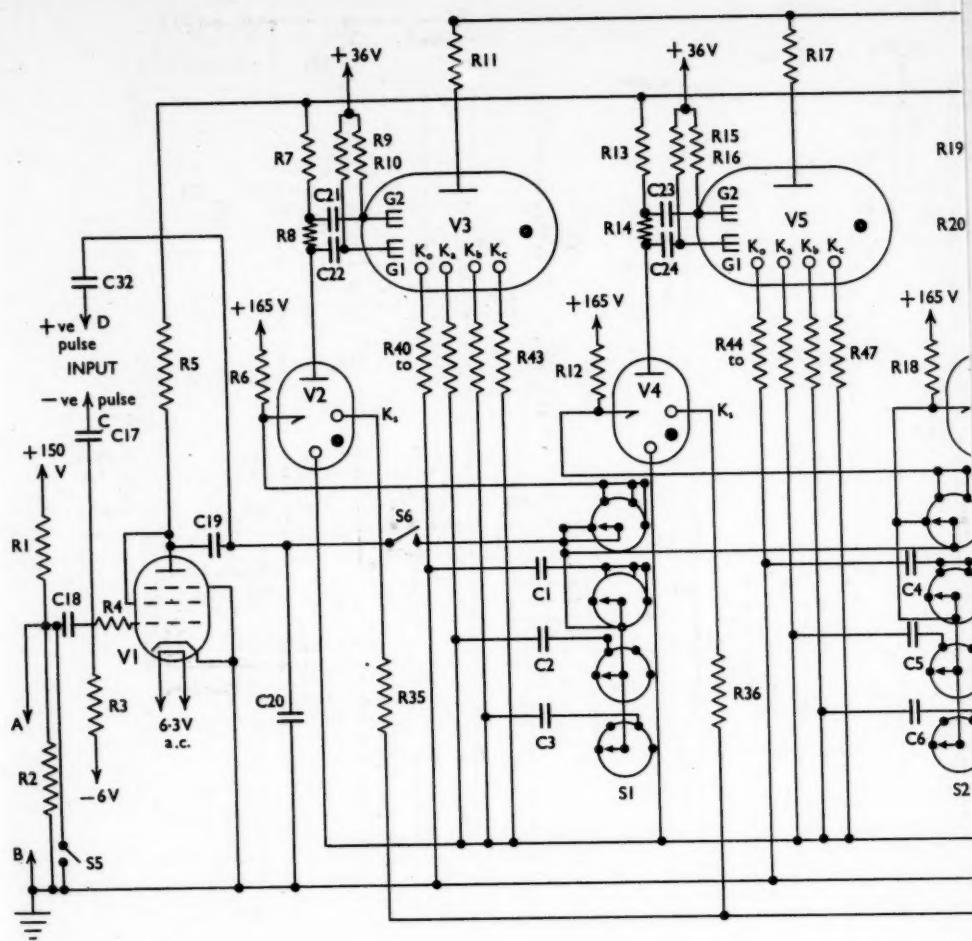
Fig. 1.   Circuit diagram of the apparatus (for va

1958

+36V   R23   +300V   +450V   R25   R31   MR 1 & 2
R21
R22
C25   G2   V7   REL. D   R24   R28   V9
C26   G   R48 to   R56 R57   H   REL. 4   +36V P1 ←165V +150   240V a.c.
V6   K₁   I   R29   V10
EK₀K₉K₈K₇...K₁   R58   E F   C29 C30
R26   V8   S8   R32 MR 3   T
C7   REL.B   S3 REL. C
C8   R33   R38 R39 6·3V a.c.
C9   R34   R30   Pilot light
R37   R27 C31   6·3V a.c. S4 REL. −6V
C10   −6V   A
RESET LINE   S7
−100V

Duodecal

K5   K4
K6   K3
K7   K2
K8   K1
K9   G2
K0   V3. V5. V7   G1
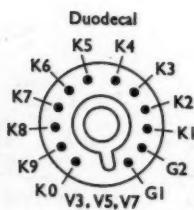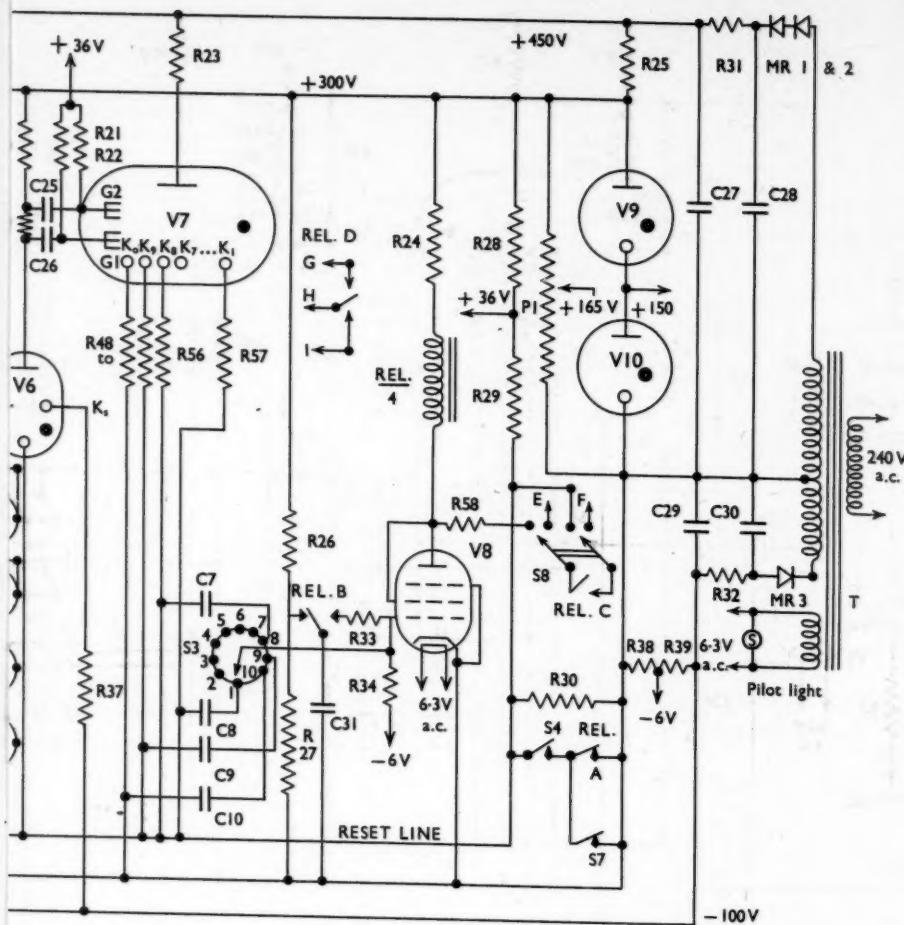
(values of components, see Appendix, p. 320)

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A Sample-injection Method for Gas - Liquid Chromatography

By F. G. STANFORD

(*U.K. Atomic Energy Authority, The Radiochemical Centre, Amersham, Bucks.*)

A method is described for the injection of accurately known weights of sample. It is of particular value when the amount of sample available is limited.

THE most usual method of placing a liquid sample on a gas - liquid chromatographic column, by means of a hypodermic syringe via a vaccine cap, necessitates filling the volume between the needle tip and the end of the plunger with sample. Any attempt to use a syringe containing air results in uncontrolled delivery of the sample when the vaccine cap is punctured, if the column pressure is below atmospheric. To charge the syringe properly, liquid is drawn into the barrel, the syringe is inverted and the air is driven out, a few drops of the liquid also being expelled.

There are several objections to this procedure when the samples are chemically toxic or radioactive, *e.g.*, labelled compounds. First, a not inconsiderable volume of sample is required, which may pose problems of screening and is certainly extravagant with expensive materials. Secondly, the ejection of a few drops from the needle tip to ensure complete expulsion of air may spread contamination. Lastly, the syringe may be difficult to decontaminate from radioactivity.

In view of the difficulties encountered when chemically toxic or radioactive compounds were introduced by the standard method into a certain commercial gas-chromatographic apparatus, the possibility of an alternative method of sample introduction was examined.

The device evolved has been so successful, particularly for limited amounts of sample, during the 8 months it has been in operation that it is thought worthy of description.

The apparatus permits the injection of accurately known weights[1] of liquid samples or solutions without the disadvantages of the syringe method already mentioned. This greatly facilitates quantitative analysis, since direct standardisation can be carried out and the result can be found from the ratio of peak size to weight, so avoiding the tedious and limited "marker" and "bracketing" methods.[1] Further, the sample is introduced to the column packing as a "plug"; this avoids the partial evaporation that may occur when a drop falls from a hypodermic needle or during injection by micrometer-syringe pipette. The complete pre-evaporation that occurs with complicated bulb-crushing and by-pass devices[2,3,4] is also avoided. Exponential flow and broadening of peak bases, which result from pre-evaporation, are therefore avoided and resolution of sample components is improved.

A great advantage when toxic or radioactive samples are used is that the injection capillaries are discarded after one injection, unlike the pipette used by Tenney and Harris.[5]

Experiments show that retention values are reproducible and correspond exactly with those obtained when a syringe is used. Sample weights from 1 to 100 mg can be used, and a graph of peak size against sample weight is linear.

DESCRIPTION OF APPARATUS

The apparatus is shown in Fig. 1. A rubber stopper, A, fitted with a glass delivery tube of bore 6 to 7 mm terminating in a 1-mm bore jet, replaces the vaccine cap that normally closes the top of the column. The jet is about 1 cm above the column packing, and the upper end of the delivery tube is closed by rubber stopper B_1. The jet serves to restrict the volume of air admitted when the column, which has an inlet pressure less than atmospheric, is opened and also as a guide when the sample capillary is inserted.

The sample capillary is 10 to 12 cm long and has a bore of 1·5 mm; one end is drawn into a jet 1·5 to 2·0 cm long and sufficiently fine to pass through the jet on the delivery tube. The wide end of the capillary is closed by a micro rubber bulb (obtained from the British Drug Houses Ltd. for use with pH capillator outfits) and fitted with a rubber stopper, B_2,

the same size as B_1 and set so that, after insertion, the capillary tip touches the column packing. The capillaries are too fragile to push through a vaccine cap.
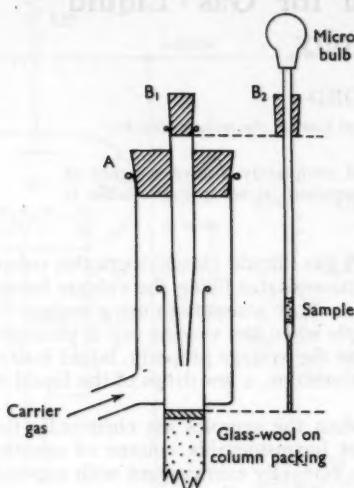

Fig. 1. Injection apparatus
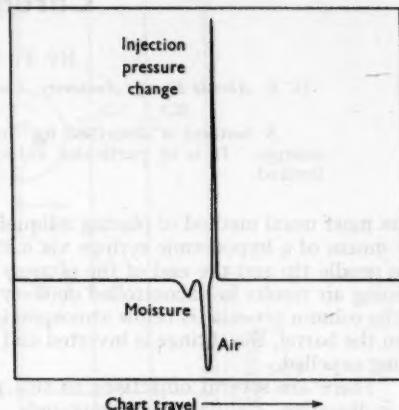
Fig. 2. Recorder chart showing peaks produced when column of Celite and silicone E301 was momentarily opened to the atmosphere

METHOD

PROCEDURE—

With the inlet closed by stopper B_1, adjust the column conditions to the required values. Prepare a capillary, and draw in the sample through the jet by means of a micro bulb; leave a space at the tip to avoid possible expulsion of sample during weighing. Wipe the jet, lay the capillary assembly on a balance pan with the jet clear of the edge, and weigh. (The capillary, together with bulb and stopper B_2, weighs approximately 1·4 g and can be conveniently weighed on a semi-micro balance.) Remove stopper B_1, immediately insert the capillary and stopper B_2, and press the bulb to expel the sample. Leave the capillary in position until the run has been completed, and then cool and re-weigh it; re-close the column with stopper B_1.

Note that no sample can be withdrawn from the capillary by reduced column pressure until stopper B_2 has been pressed home and the capillary tip is touching the packing.

RESULTS

The chart diagram obtained when the column is momentarily opened to atmosphere by removing and replacing stopper B_1 is identical with those obtained when it is opened to inject a sample and when the capillary and stopper B_2 are removed at the end of a run. Fig. 2 shows clearly the initial peak caused by pressure change and the two negative peaks produced by air and moisture.

I thank Mr. D. A. Lambie for his encouragement and advice.

REFERENCES

1. Keulemans, A. I. M., *in* Verver, C. G., *Editor*, "Gas Chromatography," Reinhold Publishing Corporation, New York, 1957, pp. 32 and 33.
2. ——, *op. cit.*, p. 67.
3. Dimbat, M., Porter, P. E., and Stross, F. H., *Anal. Chem.*, 1956, **28**, 290.
4. Davis, R. E., and McCrea, J. M., *Ibid.*, 1957, **29**, 1114.
5. Tenney, H. M., and Harris, R. J., *Ibid.*, 1957, **29**, 317.

Notes

THE DETERMINATION OF PHOSDRIN IN VEGETABLES

THE insecticide Phosdrin is a mixture of the *cis*- and *trans*- isomers of 2-methoxycarbonyl-1-methyl-vinyl dimethyl phosphate.

Although it is rapidly decomposed in plant tissues, it has high mammalian toxicity, and circumstances might arise in which its determination is necessary.

Laws and Webley's method[1] for determining demeton-methyl by the photometric measure-ment of orthophosphoric acid after a chromatographic separation may be applied to Phosdrin without modification, except that, in the evaporation of the chloroform extract, care must be taken not to lose the insecticide by volatilisation.   Removal of the solvent by gentle heating in a current of air suffices.

The Phosdrin used as standard was supplied by Shell Petroleum Company Ltd. and was 98 per cent. pure.   When a dilute solution of the insecticide was added to raw cabbage, recoveries by the analytical procedure were about 90 per cent.

Field tests were carried out on sugar-beet and kale with the active co-operation of the Plant Pathology Laboratory, Harpenden, and the Rothamsted Experimental Station.   The vegetables were sampled at intervals, as shown in Table I, and were immediately analysed for Phosdrin. Some check analyses by anticholinesterase measurement were made on the same sample solutions as were used for the chemical determination.   Attempts to keep samples in a refrigerator and to carry out the analysis at a later date showed that Phosdrin was decomposed by the plant material. On the other hand, storage in deep-freeze was satisfactory and some figures are shown for samples so treated.

TABLE I

FIELD TESTS ON SUGAR BEET AND KALE

Time of sampling after spraying, hours	Amount of Phosdrin found by—		Amount of Phosdrin found after 1 to 2 months in deep-freeze by—	
	chemical method, p.p.m.	anticholinesterase measurement, p.p.m.	chemical method, p.p.m.	anticholinesterase measurement, p.p.m.
Samples of sugar-beet leaves—				
1½	3·7	5·8	6·4	—
4½	3·6	5·5	3·8	—
21	1·05	1·1	1·3	0·97
26	1·04	0·9	1·3	—
44½	1·30	—	0·89	0·28
49½	1·40	0·9	0·69	—
68½	1·03	0·2	1·36	0·0
168	0·30	—	—	—
Control	0·03	—	—	—
Samples of kale—				
1½	5·8	6·7	3·2	—
4½	4·2	6·0	5·3	—
21	2·0	1·7	1·9	—
26	1·6	1·8	2·0	—
44½	1·4	—	2·0	0·7
49½	2·2	1·8	2·4	—
68½	1·1	1·1	1·07	0·7
168	0·5	0·7	—	—
Control	0·04	—	—	—

Phosdrin, with the addition of a wetting agent, was applied on October 21st, 1958, at a rate of 4 ounces per acre by hand-spraying.   The early samples had drops of the insecticide solution on the leaves when taken from the growing crop.   A rain-storm in the night of October 21st removed the excess of material, and the figures from 21 hours onward represent only insecticide taken up by the plants.   The anticholinesterase figures are based on the original mixture of isomers used.

In view of their different activities, it is probable that the more active isomer is decomposed before the less active, and so the anticholinesterase method tends to give lower results in the later stages.

I thank my colleagues, who carried out the analyses, and the Government Chemist for permission to publish this Note.

REFERENCE

1. Laws, E. Q., and Webley, D. J., *Analyst*, 1959, **84**, 28.

DEPARTMENT OF THE GOVERNMENT CHEMIST
    CLEMENT'S INN PASSAGE, STRAND   E. Q. LAWS
        LONDON, W.C.2   Received *January 19th*, 1959

THE ABSORPTIOMETRIC DETERMINATION OF GERMANIUM WITH PHENYLFLUORONE

THE absorptiometric method developed by Cluley[1] for determining germanium and used with slight modification in this laboratory[2] has the advantage that interference by arsenic, which can only be quantitatively separated from germanium with difficulty, is not appreciable.

Small amounts of chlorine, however, intensify the colour of phenylfluorone solution. No substance capable of oxidising hydrochloric acid must therefore be present when germanium is distilled from the fairly concentrated hydrochloric acid solution.

The experience described below shows that, although the sample may be free from such interfering oxidising agents, they might be formed during the initial stages of the prescribed procedure.

When the germanium contents of certain samples of coal were determined, the results fluctuated considerably. The green colour of the sodium carbonate melt and the faint odour of chlorine during the distillation of the germanium focused attention on manganese, of which a comparatively high concentration was found in the coal. The manganese, it was argued, is converted to manganate during the alkali fusion, and this manganate or permanganate and the manganese dioxide formed from it on acidification could have oxidised the hydrochloric acid during distillation. The resulting chlorine, which distilled with the germanium, could have been responsible for the fictitious results.

If this supposition is correct, interference by chlorine could be prevented by adding ferrous sulphate or sodium oxalate before distillation of germanium. Table I shows the amounts of germanium found in coal by Cluley's method[1] and by the modified method,[2] both in presence and absence of added ferrous sulphate or sodium oxalate. It can be seen that the results for germanium after one or other of the reducing agents had been added are only a fraction of those found without the additive. As small amounts of chlorine deepen the colour of phenylfluorone solutions and the hydrochloric acid distillates of the samples had a faint smell of chlorine, it was suggested that the lower values were correct.

TABLE I

AMOUNTS OF GERMANIUM FOUND IN COAL SAMPLES OF HIGH MANGANESE CONTENT

Sample No.	Ash content, %	Manganese content, as Mn_3O_4, %	Amount of germanium found by Cluley's method[1]—			Amount of germanium found by modified method[2]—		
			with nothing added, p.p.m.	in presence of ferrous sulphate, p.p.m.	in presence of sodium oxalate, p.p.m.	with nothing added, p.p.m.	in presence of ferrous sulphate, p.p.m.	in presence of sodium oxalate, p.p.m.
1	21·7	0·093	26·2	2·6	2·6	27·8	2·5	2·6
			27·4	2·4	2·6	31·0	2·3	2·4
2	13·0	0·023	9·0	1·8	2·0	5·4	1·7	1·9
			5·2	1·8	1·9	6·4	1·9	1·7
3	10·9	0·019	9·2	1·9	1·9	3·1	1·8	1·8
			9·0	2·0	1·8	4·4	1·8	2·0

This assumption was confirmed by further experiments to determine the influence of the manganese content of a sample on the amount of germanium found by Cluley's method. Different amounts of manganese sulphate solution were added to two coal samples, and, after the samples had been dried and mixed, their germanium contents were determined in presence and in absence of ferrous sulphate. The results in Table II show that, when no reducing agent was added, increase in the manganese content of the sample was accompanied by an increase in the amount of

germanium found. However, when ferrous sulphate was added, the manganese content of the sample had no influence on the amount of germanium found. Table II also shows that, when no

TABLE II

EFFECT OF MANGANESE CONTENT ON AMOUNT OF GERMANIUM FOUND IN COAL

The manganese content of each sample, as Mn_3O_4, was 0·004 per cent.

Sample No.	Ash content, %	Amount of manganese added, as Mn_3O_4, %	Total manganese present, as Mn_3O_4, %	Amount of germanium found by Cluley's method[1]—	
				with nothing added, p.p.m.	in presence of ferrous sulphate, p.p.m.
4	13·9	Nil	0·004	4·1, 3·8	3·4, 3·2
		0·014	0·018	5·4, 6·4	3·3, 3·3
		0·070	0·074	16·2, 19·4	3·3, 3·2
5	10·9	Nil	0·004	22·0, 22·4	21·1, 20·8
		0·014	0·018	27·4, 23·6	21·1, 20·8
		0·070	0·074	30·8, 31·6	21·3, 20·8

manganese was added, the amount of germanium found in absence of a reducing agent was somewhat high. This may be caused by either the 0·004 per cent. of manganese present in the coal or possibly by the presence of a trace of vanadate. Schneider and Sandell,[3] who also used phenylfluorone for determining germanium, observed that vanadate interfered by oxidising hydrochloric acid. They prevented the interference by adding ferrous sulphate. The reason for the slightly high germanium results was not further investigated. However, this observation led to a further modification to the procedure for germanium in that, whatever the manganese content of the sample, 1 ml of 5 per cent. ferrous sulphate solution was to be added to the solution in the distillation flask immediately before the addition of hydrochloric acid. This amount of ferrous sulphate (0·05 g of $FeSO_4.7H_2O$) represents a considerable excess, even for extraordinarily high manganese contents; it thus prevents with certainty any interference by oxidation of hydrochloric acid.

Ferrous sulphate is to be preferred to oxalate as reducing agent because it reduces not only the higher-valent manganese and vanadium, but also the direct cause of the interference, *viz.*, chlorine. The use of oxalates for this purpose was therefore abandoned.

We thank the Fuel Research Board for permission to publish this Note.

REFERENCES

1. Cluley, H. J., *Analyst*, 1951, **76**, 523.
2. Kunstmann, F. H., and Hamersma, J. C., *J. Chem. Metall. Min. Soc. S. Afr.*, 1955, **56**, 11.
3. Schneider, W. A., jun., and Sandell, E. B., *Mikrochim. Acta*, 1954, 263.

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Received *July* 14th, 1958

Ministry of Agriculture, Fisheries and Food

STATUTORY INSTRUMENTS*

1958—No. 2167. The Public Health (Preservatives etc. in Food) (Amendment No. 2) Regulations, 1958. Price 3d.

These amending regulations, which came into operation on December 24th, 1958, provide for the sale and importation of apples, pears, pineapples, peaches and melons, and articles of food containing any of these fruits, which contain ortho-phenylphenol within prescribed limits. (Citrus fruits were dealt with in S.I. 1958, No. 1319, mentioned in Analyst, 1958, **83**, 591.)

1959—No. 471. The Labelling of Food (Amendment) Regulations, 1959. Price 3d.

These regulations, which came into operation on April 27th, 1959, amend the Labelling of Food Order, 1953 (S.I. 1953, No. 536) as amended, by adding provisions relating to ice-cream.

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

1959—No. 472. The Food Standards (Ice-Cream) Regulations, 1959. Price 3d.

> *These regulations, which came into operation on April 27th, 1959, prescribe amended standards of composition for ice-cream and "Parev" (Kosher) ice and introduce standards for dairy ice-cream and milk ice.*

Book Reviews

THE DETERMINATION OF NITROGEN IN STEEL. Special Report No. 62 of the British Iron and Steel Research Association. Pp. xii + 146. London: The Iron and Steel Institute. 1958. Price 37s. 6d.

A study group was set up in 1949 by one of the Technical Committees of B.I.S.R.A. with the following terms of reference—"To review the position with regard to the chemical determination of nitrogen in steel; to examine the reproducibilities and general applicabilities of the methods in use; to recommend one or more standard methods and to compare the chemical and the vacuum fusion methods." This report gives the results of the group's work.

In Part I are given the results of detailed investigations into the various factors affecting the quantitative recovery of nitrogen from plain and alloy steels. In particular, two groups of alloying elements have been identified; those that can lead to the formation of acid-resistant nitrides and those that do not. It is shown that the dissolution of the sample is the most critical step in the procedure and that a choice of methods is permissible for the subsequent determination of the ammonia.

A standard method is proposed, applicable to all types of steel, but it is pointed out that complete recovery of nitrogen cannot be obtained from steels containing silicon nitride. The method has been submitted to and accepted by the British Standards Institution.

Part II deals with the vacuum fusion method of analysis. The results show that there can be excellent agreement between the chemical and the vacuum fusion methods. They are also considered to refute suggestions that the chemical method always gives higher results for nitrogen.

These two parts of the report cover the first 50 pages; the remainder consists of appendixes and tables of results. There is also a section giving results of a separate study of the chemical determination of nitrogen in ferro-chromium.

The report contains much valuable information and represents a considerable amount of co-operative work carried out over a long period. During the 10 years since the work was started the use of vacuum fusion techniques has greatly increased, and it might have been appropriate to give more details of the techniques used in the different laboratories, as has been done for the chemical methods. A similar comment applies to the methods of gas analysis employed; why need they be "outside the scope of this report"?

It is to be hoped that a study of this report will break down any mental barriers, if they remain, between the devotees of "wet" methods of analysis and those of the newer physical techniques. All are now in the province of the analyst.   R. C. CHIRNSIDE

ANALYTICAL CHEMISTRY: SOME NEW TECHNIQUES. By A. G. JONES, B.Sc., F.R.I.C. Pp. viii + 268. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1959. Price 40s.; $7.50.

In the first place it is important to realise that the author of this book has had a particular purpose in mind. He has aimed to supply senior members of the analytical profession with an introduction to some of the more important procedures in analytical chemistry that have been developed in recent years, dealing particularly with a review of the progress in these subjects between 1950 and 1957. He has selected eight topics of special interest to himself; from the reading of the book it is obvious that he has either had personal experience in the fields covered or, alternatively, he has worked in a laboratory where these methods have been used. The subjects chosen include differential spectrophotometry, differential refractometry, flame photometry, gas chromatography, the use of ion-exchange resins in analytical chemistry, acid - base titrations in non-aqueous media, coulometric titrations and the determination of oxygen and hydrogen in metals. Each chapter is complete in itself and usually includes a simple description of the relevant procedure, the type of equipment used and practical examples of the application of the method. A strong feature of the book is the inclusion of adequate references to each chapter. Moreover, these references are the right ones, because it is obvious that the author has studied them over and

over again.   There is little point in making minor criticisms of this book, because the proof reading has been well done by a panel of experts.   Senior analysts, for whom this book is intended, will read it with interest and profit.

Perhaps it might have been better either to produce each chapter as a monograph or, alternatively, for the author to have persuaded his able colleagues to join him in producing, on the same lines, a much more comprehensive account of the numerous developments in analytical chemistry during the last 10 years.   J. HASLAM

Publications Received

THE PRINCIPLES OF ELECTROPHORESIS.   By RENE AUDUBERT and SERGE DE MENDE.   Translated by A. J. POMERANS.   Pp. viii + 142.   London: Hutchinson & Co. (Publishers) Ltd. 1959.   Price 25s.

MONOGRAPHIEN AUS DEM GEBIETE DER QUALITATIVEN MIKROANALYSE.   Edited by A. A. BENEDETTI-PICHLER.   Volume I.   ANORGANISCHE QUALITATIVE MIKROANALYSE.   By HANNS MALISSA and A. A. BENEDETTI-PICHLER.   Pp. viii + 333.   Wien, Austria: Springer-Verlag.   1958.   Price DM 49; sfr. 50.20; $11.65; 83s. 6d.

TRANSLATION FROM GERMAN FOR CHEMISTS.   By H. H. NEVILLE, M.A., B.Sc., Ph.D., and W. E. YUILL, M.A.   Pp. xii + 139.   New York: Interscience Publishers Inc.; London and Glasgow: Blackie & Son Ltd.   1959.   Price 15s.

TROPICAL SCIENCE.   Edited by R. H. KIRBY, B.Com., Ph.D.   Volume I, Number I, 1959.   Pp. iv + 70.   London: Her Majesty's Stationery Office.   Price 6s.

   Successor to Colonial Plant and Animal Products.

JOURNAL OF BIOCHEMICAL AND MICROBIOLOGICAL TECHNOLOGY AND ENGINEERING.   Edited by E. M. CROOK, M. B. DONALD and ELMER L. GADEN, JUN.   Volume I, Number I, February, 1959.   Pp. 127.   New York and London: Interscience Publishers Inc.   Annual Subscription $15.00; 115s.

   A new journal.

EDTA TITRATIONS: AN INTRODUCTION TO THEORY AND PRACTICE.   By H. A. FLASCHKA, Ph.D. Pp. 138.   London, New York, Paris and Los Angeles: Pergamon Press Ltd.   1959. Price 42s.

JOURNAL OF MEDICINAL AND PHARMACEUTICAL CHEMISTRY.   Edited by ARNOLD H. BECKETT and ALFRED BURGER.   Volume I, Number I, February, 1959.   Pp. 120.   New York and London: Interscience Publishers Inc.   Annual Subscription $15.00; 115s.

   A new journal.

THE TRANSURANIUM ELEMENTS.   By GLENN T. SEABORG.   Pp. xxii + 328.   London: Methuen & Co. Ltd.   1958.   Price 50s.

A TEXT-BOOK OF BACTERIOLOGY.   By R. W. FAIRBROTHER, T.D., M.D., D.Sc., F.R.C.P.   Eighth Edition.   Pp. viii + 502.   London: William Heinemann Medical Books Ltd.   1959. Price 25s.

CRYSTAL STRUCTURES.   Supplement IV.   By RALPH W. G. WYCKOFF.   Loose-leaf, 394 sheets. New York and London: Interscience Publishers Ltd.   1959.   Price $22.00; 165s.

   Supplement IV consists of punched leaves for insertion into chapters IX, X, XIII, XIV and XV.

NOMENCLATURE OF INORGANIC CHEMISTRY.   Definitive Rules issued by the Inorganic Chemistry Section of the International Union of Pure and Applied Chemistry.   Pp. x + 93. London: Butterworths Scientific Publications.   1959.   Price 15s.

   This volume contains the 1957 report of the Commission on the Nomenclature of Inorganic Chemistry.   English and French texts appear side by side.

THE SOCIETY FOR ANALYTICAL CHEMISTRY

FORMERLY THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

FOUNDED 1874.   INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry by holding periodical meetings, by promoting lectures, discussions and conferences, and by the publication of a journal devoted to all branches of analytical chemistry; to study questions relating to the analysis, nature and composition of natural and manufactured materials generally; and to promote, or assist to promote, the efficiency and the proper administration of the laws relating to the control and composition of such materials.

The Society includes members of the following classes:—(a) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (b) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society who shall provide written testimony of their personal knowledge as to his scientific and professional fitness. If the Council in their discretion think fit, such testimony may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to elect candidates or to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so wishes. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings, but are not entitled to vote.

The Entrance Fee for Ordinary Members is £1 1s. and the Annual Subscription is £3 3s. Junior Members are not required to pay an Entrance Fee and their Annual Subscription is £1 1s. No Entrance Fee is payable by a Junior Member on transferring to Ordinary Membership. The Entrance Fee (where applicable) and first year's Subscription must accompany the completed Form of Application for Membership. Subscriptions are due on January 1st of each year.

Scientific Meetings of the Society are usually held on the first Wednesday in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. The Annual General Meeting is usually held in London on the first Friday in March. Notices of all meetings are sent to members by post.

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

The Analyst, the official organ of the Society, is issued monthly, to all Ordinary and Junior Members, and contains reports of the proceedings of the Society, original papers and notes, information about analytical methods, Government reports and reviews of books. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

LOCAL SECTIONS AND SUBJECT GROUPS

THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas. The Microchemistry, Physical Methods and Biological Methods Groups have been formed within the Society to further the study of the application of microchemical, physical and biological methods of analysis. All members of the Society are eligible for membership of the Groups.

The Sections and Groups hold their own meetings from time to time in different places. There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary.